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GENETICS

A Periodical Record of Investigations Bearing on Heredity and Variation

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THE BLOOD RELATIONSHIPS OF FOUR STRAINS OF MICE

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Received September 3, 1930

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I. PRECIPITATING ANTISERA BUILT UP IN PIGEONS

Object

The extensive survey of the animal kingdom made by NUTTALL by means of precipitating antisera is complete except for the relationship of the order Rodentia. Our object in this work was to develop a standard method for the production of an anti-rodent precipitating serum and by it to study the blood relationship of the several strains of mice available in

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our laboratory. We hoped to determine the origin of the Japanese waltzing mouse and of *Mus bactrianus*.

Technique of production

Choice of materials and method

Since ordinary methods of immunization have been considered unsuited for the production of rodent antisera the choice of animals for the reception of the antigen was very difficult. GATES considered that the rabbit was unsuited, after a preliminary attempt to immunize two. NUTTALL believed that the rabbit might reasonably be considered to possess natural antibodies for the serum of another rodent and as a consequence he attempted the immunization of the chicken and the duck by rodent protein, failing in both instances. He suggests that the most desirable type of animal would be the goat or the eel. This conclusion is doubtless based on his extensive research on the relations of various animal sera. He believes that an animal far removed phylogenetically from the one to be tested is the most desirable as the antibody producer, and in addition he states that in his first work he followed NOLF in this particular. NOLF, in 1900, produced an antibody for rabbit serum in a fowl. BORDET (1899) and HAMBURGER (1902) found that guinea pigs were unsuited for the production of rodent precipitin, and it is generally considered true that the guinea pig is not a desirable species to employ for antibody production. GENGOU, in 1900, described a distinct opalescence as occurring in the test of serum from a guinea pig immunized with rabbit serum, but this opalescence did not go on to the formation of a precipitin.

In spite of NUTTALL'S suggestion in favor of goat or eel we decided to try the pigeon and chicken as antibody producers because the care of goats and eels is a problem and the mode of treatment for these animals perhaps even less known than is the technical procedure for pigeon or fowl. And, furthermore, insomuch as several workers have chosen birds as the antibody producers in serological experiments with rodents, we wished to be able to check the technique in these cases.

After a small amount of preliminary work we eliminated the chicken because the technique of injection proved simpler in the pigeon, and, further, the large number required for the work could be much more readily handled than the same number of chickens. Only four chickens were used in the experiment, the remainder of the inoculated animals being pigeons. These were from a standard pedigreed stock furnished us by the late Doctor METZLAAR of the UNIVERSITY OF MICHIGAN.

Preparation of antigen

Antigen No. 1 (See figure 1). Young adult mice of the highly inbred *Mus musculus*[1] carried in this laboratory were bled for the serum used as antigen No. 1. A 1 cc tuberculin luer was equipped with a rubber tube at the open

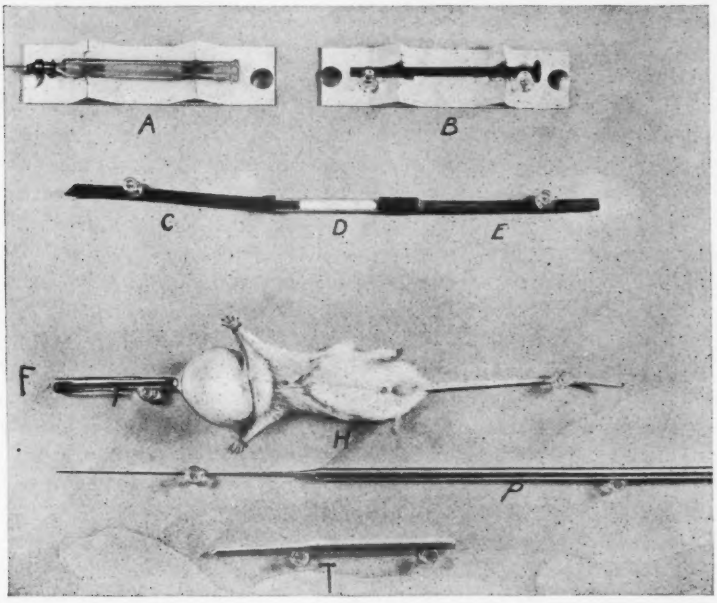

FIGURE 1.—Apparatus for cardiac puncture of mouse. Pipette and tube for precipitin test.
A-B—1 cc tuberculin luer ready for use in mouse bleeding.
C—Catheter tubing cut at angle to fit tightly in open end of syringe.
D—Short piece of glass tubing with cotton.
E—Tubing to mouth of operator.
F—Cotton in small glass anaesthetic hood.
H—Mouse ready for cardiac puncture. In practice the thumb and forefinger of the left hand replace the glass push pin shown here on the tail of the mouse.
P—Drawn pipette for filling tip of precipitin tube with the antiserum.
T—Precipitin tube.

end so that the tubing made a tight seal. The tubing was cut a short distance from the luer and a glass tube inserted, packed with sterile cotton. Many such rubber-glass-rubber tubes were made and sterilized in a wrap-

[1] The strain used was the dilute brown (db_r) stock described by LITTLE (1916) and again by MURRAY (1927).

per so that they could be quickly inserted in the end of the freshly boiled luer. With a half-inch No. 24 needle the heart of the mouse was punctured and from 0.5 to 1 cc of blood withdrawn by mouth suction exerted on the end of the rubber tube. (It is difficult to blow the blood from the syringe, but if the rubber tube is quickly removed and the plunger inserted the blood may be readily expelled.) The blood obtained in this way from several mice was allowed to clot in a single centrifuge tube, rimmed after thirty minutes and the serum separated by centrifugation. The animals were lightly anaesthetized, fastened on their backs to a small board and swabbed with 70 per cent alcohol prior to the cardiac puncture. Often, after the blood was withdrawn, the mouse would run off the board apparently as well as ever.This procedure was repeated on the same mice, at intervals of two weeks, as many as twelve times and the animals still seemed normal.

The chief advantage of the suction method over the one in which blood is withdrawn by a plunger is that the blood begins to flow into the luer the instant the open end of the needle enters the tiny blood space in the heart and the suction may continue longer than even the best of syringes will permit. In addition, one hand is left free to hold the animal or palpate for the xyphoid process as an aid in determining the landmarks of the anterior thoracic wall. Of the many stock amounts of serum collected in this manner there was but one which was contaminated and in no instance did we use any preservative.

Antigen No. 2: Extract of dried Mus musculus muscle tissue. No. 6: Extract of dried Mus bactrianus muscle tissue. No. 7: Extract of dried Mus faeroensis muscle tissue. No. 14: Extract of dried Japanese waltzing mice muscle tissue. The method here was the same as that used by GATES in his study of the Japanese waltzing mouse. The muscle tissue in each case was dried at 50° C for three days under aseptic conditions and at the end of that time stored in a desiccator. Fifty-five hundredths grams of this dried muscle extract were thoroughly macerated with 5 cc of physiological salt solution and the whole allowed to stand at room temperature for six hours with frequent shaking. Although the preparation in every case was as aseptic as possible no extract was considered sterile and was therefore not kept for any injection subsequent to the one for which it was specifically prepared. The extract was not filtered but the sediment was allowed to settle for ten minutes before the syringe was loaded from the supernatant fluid.

Antigen No. 3: Extract of fresh Mus musculus muscle tissue. No. 5: Extract of fresh Mus bactrianus muscle tissue. No. 8: Extract of fresh perfused guinea pig kidney. The preparation of these antigens was again by the method of GATES. One gram of macerated muscle with 5 cc of physiological

salt solution constituted the extract and the treatment was the same as for antigen No. 2.

In the case of the *bactrianus* antigen it was necessary, on account of the small amount of material available, to make up the whole extract at once and keep it in sealed ampules with a preservative of 0.25 per cent cresol. It kept in the refrigerator without apparent change as long as was required.

The extract of the guinea pig kidney was made as needed and prior to the excision of the organ it was thoroughly perfused with Ringer's solution.

Antigen No. 4: Mus musculus whole blood. This antigen was collected from the mice in the manner described for antigen No. 1 except that the tube into which it was placed after withdrawal contained two drops of a 20 percent solution of potassium oxalate dried down to crystalline powder. The blood as it was added to this tube was shaken well and the amount of the oxalate present was enough to prevent coagulation of amounts of blood up to 10 cc. In the case of injections given in group 1 of Series IV with this antigen, there was no necessity for the use of a substance to prevent clotting as the transfer was made so quickly that there was no time for a clot to form. However, in the latter part of the injections carried on in Series IV, Group 2, we had to resort to the pooling of blood to obtain the amounts needed for injection.

Antigen No. 9: Rabbit serum. This antigen was obtained by cardiac puncture. The serum was separated from the cells and stored without preservative in sealed ampules.

Antigen No. 10: Human serum of the five different races. The use of this serum was made possible by the assistance of Doctor S. I. IDE of the serological laboratory of the UNIVERSITY HOSPITAL, Ann Arbor, Michigan, who obtained the serum for us.

Antigen No. 11: Serum of Rattus rattus norvegicus. No. 12: Serum of Rattus rattus tectorum. No. 13: Serum of Rattus rattus rattus. This serum was obtained in the same manner as that for antigen No. 1, except for the fact that a 10 cc luer was used with a cork in the open end, which was pierced by a tube leading to the mouth of the operator. By this suction method it was quite simple to obtain from six to eight or 10 cc of blood from a rat and have him survive. Whereas with mice the mortality from cardiac puncture was very low, under 5 percent, with rats it was about 50 percent. This we accounted for by the fact that the degree of anaesthesia required to insure the safety of the operator was much greater with rats, and since we bled very few rats in comparison with the number of mice so treated our technique in the case of rats was much less skilled.

Production of antisera

The manner of preparation of antisera was varied from that of BEHRENS, by which 0.1 cc of antigen is given daily for ten or more days, to that of GAY and FITZGERALD, by which a rapidly increased dosage is injected every three days. Every reasonable modification of dosage was attempted. We kept one part of the method standard, however, that is, all injections were intravenous.

Intravenous injection in the pigeon or chicken is very simple until the clots about the vein become so dense that the wall cannot be seen and the lumen in some instances occluded. At this point the problem of intravenous injection becomes very great. However, there are two veins in each leg which may be used at least once each and by the time the process has gone through both wings and both legs the first one used is usually ready for another injection. We did not resort to the removal of small pieces of skin to facilitate the injection as did FINLAY.

In the beginning we considered that the suffusion of blood under the skin at the point of puncture after the removal of the needle might best be stopped by gentle pressure or the application of collodion over the spot. We learned that even if the spot was compressed for as long as 15 minutes after the removal of the needle the bleeding would be just as profuse and the hematoma just as inevitable. After the vein has been exposed by removing the feathers, and the skin washed with 70 percent alcohol, the most successful avoidance of hematoma is accomplished by simply leaving the needle in the vein for a few minutes and removing it quickly without applying any pressure at the site of the puncture. In this case the subcutaneous tissues seem to be more intact and the small drop of blood which appears as the needle is removed comes directly along the path made by the needle as it entered and hence there is no suffusion of blood around the vein. By this method we could give a bird daily injections for 20 days and still have the veins readily accessible in both wings. In fact a path seemed to be created into the vein and the old spot was better than any new one. If the technique of intravenous injection is properly worked out, and the dosage so controlled that the animal withstands foreign protein reactions successfully, the intravenous mode of administering antigen is the best. We feared at first that we might occasionally be forced to intramuscular injection in the pigeon and relied upon the rich capillary network of the breast muscle to take up such injection, but the method for the intravenous administration of antigen was so successful that no other type was ever given.

The pigeons were weighed daily throughout the course of treatment, on the assumption that if they lost weight markedly it would be advisable to modify the dosage. The weight in all instances however remained within the normal limits of variation explainable by differences in the food or feeding hour. Since it is possible that the weight loss of an animal may be a function of his antibody production, it is notable in this work that, antibody production being very poor, the weight of the animals did not seem to be affected.

Collection of antisera

In every instance we did preliminary titration of each antiserum as often as was feasible in the hope that we could catch the curve of antibody production at its height and bleed the bird out at that time. In a few instances the slight amount of precipitin obtained was seen to disappear quite quickly, that is, within three days, from the blood of the bird.

The serum from these preliminary titrations was obtained by cardiac puncture, again using the method of suction by mouth in preference to the withdrawal of the plunger of the syringe. It was possible with the use of an inch and one-half No. 20 needle to pierce the breast bone of the lightly anaesthetized pigeon and draw 5 to 10 cc of blood very readily. It is also possible to go down through the angle of the breast bone along the line of the trachea and obtain blood from the heart but such a mode of approach gives rise to a considerable mortality: the presence of the big vessels and the thinner wall at the base of the heart make it a dangerous site for the introduction of a needle and in addition the trachea may be pierced on this route and the bird drown in its own blood. We therefore abandoned it in favor of a direct plunge through the pectoral muscles and breast bone. In this latter method the needle may be made to enter the ventricles. The hole heals readily in the thick muscle unless a coronary artery is pierced, in which case death is immediate from tamponading of the heart by an hemopericardium.

For preliminary bleeding a 10 cc syringe was used but when the animal was bled out for the last titrations for specificity of antibody a 50 cc syringe was substituted. The maximum of blood which could be obtained at a single bleeding was found to be 35 cc from a bird weighing about 350 grams.

Since precipitating antisera must not be chylous or opalescent the birds were starved for 24 hours before the bleeding for preliminary titrations and sometimes for longer periods in the case of the final bleeding, for even after 24 hours the serum was apt to be chylous if food could be felt in the crop.

Considering the controls employed in the precipitin test we could allow for slight opalescence in the preliminary titrations, but in no instance did we need to concern ourselves in the final titrations with doubtfully clear antisera.

The tenacity of the clot in pigeon blood is so great that the serum yield is small, and regardless of how carefully the blood is handled (in paraffin-lined tubes, etc.) there is some reddening of the serum upon separation either by centrifuging or by simple contraction of the clot in the refrigerator. The serum which we at first secured was deep red, and, although crystal clear, not at all suitable for precipitin work. Since this obstacle was very great we decided to risk the addition of oxalate to obtain a better yield of straw yellow serum.

To be sure that this foreign salt would not affect the precipitin we used it in four instances with high titer rabbit antisera prepared against the human and found that the titer of the serum collected in the ordinary way and the titer of that collected in the presence of enough potassium oxalate to prevent clotting, were identical. We also tried this duplication of method, that is, with and without the oxalate, on four pigeons with a titer of anti-human precipitin at 1:50, and in all instances the final reading was the same, the only difference being that the pigeon antiserum obtained by oxalating was straw yellow and clear while the other was much discolored by hemoglobin. NUTTALL notes (1904, p. 408) that the addition of increased amounts of sodium chloride does not alter a precipitin reaction and we feel safe in concluding from his observation and from our own work that the oxalate used did not inhibit any precipitating antibody which might have been developed. We consider this to be especially logical when we recall that in preliminary titrations, where smaller amounts of antisera were required, it was often unnecessary to oxalate the blood and yet there were no unaccountable discrepancies between the last preliminary titration and the final titration in which the oxalated serum was used.

To know the best time for bleeding was very difficult. We considered at the beginning that in general the antibody might be present as early as the sixth day and begin to diminish in about 10 days to two weeks. This was not the case. The antibody sometimes appeared early and left again in a few days and no amount of inoculation would bring it back again. Because of the uncertain character of the antibody formed we may not set the time of the appearance of maximum titer but we believe that, in many instances at least, it is not at the end of the ordinary 10 to 12 day interval after the last injection; it may reach its peak and begin to

decline sharply in four days after the last treatment. In general we tried to gauge the time of final bleeding by the result of the preliminary titration, but in case this was consistently negative after a 10 to 14 day rest from treatment, we either bled the bird out in the hope that titration at higher levels might bring out some type of zonal reaction or we continued treatment with the particular antigen.

Technique of precipitin test

Preparation of glassware

To make the precipitin tube, six mm glass tubing is cut into approximately 15 cm lengths and drawn to a blunt taper at the center. The taper is then cut and sealed at a constant length of about 75 mm. Care is taken that the wall of the glass tip is fairly strong. Since the freshly drawn glass has an alkaline surface which might influence a precipitin reaction, the tubes are thoroughly washed through chromic acid, sodium hydroxide and distilled water before being used.

To the bottom of the drawn tube we add the antiserum undiluted by means of an ordinary 1 cc pipette graduated in hundredths with a long, finely drawn tip that will reach to the bottom of the taper on the precipitin tube. In this way 0.2 cc of the antiserum is placed in each tube and then 0.5 cc of the antigen dilution is overlaid by means of suitable pipettes. If this is permitted to slide down the side of the tube a very distinct interface between the antiserum and the antigen dilution is formed. This is a common method used by many workers and facilitates the reading of the precipitate ultimately formed as well as the early ring test at the interface.

Dilutions of antigen

For preliminary titration of antisera dilutions of antigen were used which we considered might give us an index to the progress of the reaction in the pigeons and such dilutions ranged from 1:4 to 1:100. In the final titration the dilution of antigen in physiological salt solution began at 1:4 and was carried up to 1:5,000 in the steps given in the charts. In test dilutions for specificity of the precipitin in question the titer with heterologous serum was always determined for one or more tubes beyond the end-point of the reaction with the homologous antigen. The dilutions of antigen were prepared with 2 cc pipettes graduated to thousandths of cc. This method was outlined by DOCTOR PHILIP B. HADLEY of the Department of Bacteriology and it was at his suggestion that the dilution of antigen rather than the dilution of antiserum was used as standard.

With other physiological differences, such as higher body temperature and naturally higher resistance to almost all infection, the pigeon has a higher salt concentration in body fluids than mammals. It might be supposed that antisera collected from a pigeon might give a better precipitin reaction in the presence of antigen dilutions made with a salt concentration of more than the usual strength. With this in mind stock precipitating antisera from pigeons and rabbits were titrated against homologous and heterologous antigens diluted with salt solutions of 0.75 percent, 0.85 percent, 0.95 percent, 1.15 percent, 1.35 percent, 1.5 percent, 1.8 percent and 2.5 percent, and no difference in the end-point or the character of the reaction at any stage was observed. All routine preliminary and final titrations were accordingly done with antigen dilutions made from serum with 0.75 percent NaCl in distilled water.

Observation and reading of tests

After the antiserum was overlaid by the antigen dilution we placed the tubes at once in a water bath at 37° C and watched them for one hour for the appearance of ring tests. Record was made of the presence of a ring and then the contents of the tube were thoroughly mixed by whirling at a slant and with a sterile platinum wire. They were then given 24 hours in the refrigerator and observed several times during this period. We finally came to accept as standard only such tubes as showed a precipitin at the end of 24 hours. The ring test proved to be frequently deceptive as might well be expected from the very delicate character of interfacial reactions. In several instances antisera gave clouding at the interface and did not go on to precipitation after mixing. Fifteen minutes after the two solutions had been mixed the truly positive ring was readily determined by the presence in the tube of a finely dispersed flaky precipitin closely resembling the phenomena to be observed in a four plus Kahn reaction. As in the Kahn test the size of these particles varied considerably. In 24 hours the precipitin would invariably have settled to a small white pile at the bottom of the tube, tapering down in amount as the dilution increased. In the case of every antiserum which we considered to be positive the final reading could have been made in one hour and the test considered complete. Since BORDET has stated that he considers precipitating antisera to be definite after one hour at room temperature and uses as his standard method the dilution of antigen rather than the antiserum, the method which has been used here in the tests of rodent antisera has sufficient precedent.

Readings taken beyond the 24 hour interval with this technique are not of significance. Since the tubes are left open at the top contamination is certain to occur in that time and the growth of bacteria after 24 hours will often give a false positive reaction. Bacterial growth may be noted very rarely during the period of refrigeration but it appears as a uniform haze at the top of the tube and need not be confused with a positive precipitin test as described above.

Asepsis

All dilutions were prepared with sterile reagents and glassware, but the tubes containing the final dilutions were not plugged nor were the precipitin tubes closed to the air. There were no instances in which antisera were contaminated prior to titration.

Controls

The controls used for a single titration of antiserum against its homologous antigen were very simple. A tube of the antiserum was overlaid with physiological salt solution for the first control and the second was pre-immunization serum overlaid by a low dilution of the antigen. Every pigeon was subjected to a 4 to 6 cc bleeding prior to the start of the immunization and this small sample of serum was retained to use as a control when, later on, titrations of the induced precipitin were made. It is notable that throughout the entire work a normal pigeon serum, "fresh," or "ice box ripened," was never found to contain a natural precipitin for any rodent protein.

If the antiserum being tested was found to give a positive precipitin reaction with the particular dilution of antigen for that day, the entire series of antigen dilutions was tested with the normal, pre-immunization pigeon serum again as a further guarantee of the induced nature of the precipitin observed.

Antigen index

I—*Mus musculus*—serum.
II—*Mus musculus*—extract of dried muscle tissue.
III—*Mus musculus*—extract of fresh muscle tissue.
IV—*Mus musculus*—whole blood.
V—*Mus bactrianus*—extract fresh muscle tissue.
VI—*Mus bactrianus*—extract dried muscle tissue.
VII—*Mus faeroensis*—extract dried muscle tissue.
VIII—Guinea pig kidney—extract of fresh perfused kidney.

IX—Rabbit—serum.
X—Human serum. Five races.
X–A, Caucasian
X–B, Ethiopian
X–C, Indian
X–D, Mongolian
X–E, Malay
XI—*Rattus rattus norvegicus*—serum.
XII—*Rattus rattus tectorum*—serum.
XIII—*Rattus rattus rattus*—serum.
XIV—Mus (Japanese waltzer), extract dried muscle tissue.
XV—Horse—serum.
XVI—*Mus musculus*—organ transfer (antigen OT).

Data—presentation and comment.[2]

With antigen No. 1, *Mus musculus* serum, nineteen pigeons were immunized. Three pigeons received injections, on the first, third and fifth days, of 1.0, 3.0 and 5.0 cc of the antigen. In addition, four groups of four birds each were treated as follows: first group, 0.1 cc daily for ten days; second group, 0.1 cc daily for twenty days; third group, 0.1 cc daily for ten days, seven days rest, repeat 0.1 cc daily for ten days; fourth group, injected every three days in doses regularly increased by 0.25 cc, from 0.25 cc to 2.5 cc, in ten injections. A single bird was added to each group and received physiological salt solution as a control injection.

Six birds in all gave positive precipitating antisera. There were no significant differences referable to the method of immunization. The highest reaction obtained was at an antigen dilution of 1:40 and that was entirely non-specific among the four mice being studied, *Mus norvegicus*, and the rabbit. This antiserum also reacted with human serum at dilution of 1:10. The other five antisera giving positive results were nonspecific among the rodents but differentiated human serum. The results of these titrations were consistent in four preliminary titrations and the final tests for specificity.

Using antigen No. II, an extract of dried muscle tissue from *Mus musculus*, made according to the method of GATES, five birds were treated by injections given four days a week for two weeks in doses graduated from 0.5 to 3.0 cc. In addition, a control bird was given similar injections of

[2] The original tables of all preliminary and final titrations of antisera obtained, with dates and intervals for all injections and subsequent bleedings, are omitted here for the sake of brevity, but they are available on request to anyone wishing to study them.

physiological salt solution. The inoculum was of too high viscosity and was poorly tolerated when injected intravenously. No precipitin was obtained.

Antigen No. III, a fresh muscle extract from *Mus musculus*, was found dangerous for intravenous administration unless the supernatant fluid was free of particles. This antigen was given to three groups of pigeons: first group, two birds, 0.1 cc daily for twenty days; second group, five birds, injected on alternate days with doses increased regularly by 0.5 cc, from 0.5 cc to 2.5 cc; third group, three birds, injected four days a week for two weeks in doses graduated from 0.5 to 3.0 cc. There was also one physiological salt solution control bird in each group. None of the birds treated with this antigen gave any precipitating antibody in its serum.

Nine birds were immunized with antigen No. IV, *Mus musculus* whole blood. Four received 0.1 cc daily for ten days; five received injections increased by 0.1 cc per day from 0.1 to 1.0 cc. One bird in each group gave positive reactions at dilutions of 1:20 with homologous antigen, and another in the second group gave a reaction at 1:12. Both positive sera in the second group showed a degree of group specificity in so much as they did not react at such high titer with rat, rabbit or human serum. If the difference observed in the tests for specificity of these antisera had been consistent and had occurred in dilutions near 1:500 these might have been considered dependable precipitating antisera. In the first tests the ring phenomena were thought to be positive in dilutions up to 1:800 of homologous antigen, but since the clouding at the interface did not go on to formation of a definite precipitin after the antigen and antisera were thoroughly mixed the ring test was considered to be a false one.

With this antigen, *Mus musculus* whole blood, five birds were lost in an early experimental effort to determine the limit of tolerance of the antigen. A pigeon, 300 to 380 g, became dyspnoeic and lost muscular coördination after the injection of 1.5 cc of mouse whole blood. Amounts over 1.5 cc would cause death.

Five pigeons were treated with antigen No. V, an extract of fresh muscle from *Mus bactrianus*. The first received 1.0 cc daily for ten days; the second, 1.0 cc daily for fifteen days; the third, 1, 2, 3, 4, 5, and 6 cc at two-day intervals; and the fourth and fifth received 1.0 cc daily for ten days, seven days rest, repeat 1.0 cc daily for ten days. The first bird gave a very low titer precipitin, non-specific within the rodent group. The remaining four birds developed no precipitating antibody.

Six pigeons similarly treated with antigen No. VI, an extract of dried muscle tissue from *Mus bactrianus*, gave no precipitin.

Seven pigeons similarly injected with antigen No. VII, an extract from the dried muscle of *Mus faeroensis*, gave no precipitin. Four control birds given similar doses of antigen No. VIII, an extract of fresh, perfused guinea pig kidney, were also negative.

In a control series, antigen No. IX, normal rabbit serum, was given to twelve birds. The first group, five birds, received 1.0 cc daily for ten days; second group, three birds, received 1.0 cc daily for ten days, rest seven days, repeat 1.0 cc daily for ten days; third group, four birds, was injected every third day in amounts increased by 0.5 cc at each injection with a range from 1.0 cc to 3.0 cc. Three pigeons in the first, two in the second, and three in the third group gave positive antisera.

Pigeon No. 90, first group, titrated on November 27, 1927, gave a precipitin at 1:4 dilution of homologous antigen (rabbit serum) but was negative at 1:20. When the bird was bled out two days later the titer was found to have risen to 1:20. A repeat titration of the serum reserved from the bleeding November 27 showed a check titer at 1:4 and no reaction above that point. Duplication of the titer first obtained on the serum drawn November 29 was obtained so that we have indication that the serum markedly increased its titer in two days. Although the increase in titer from 1:4 to 1:20 is not significant in the type of precipitating antisera usually obtained from rabbits, it is significant in the case of pigeons where the average titer obtained under any treatment employed here was less than 1:50.

Although a titer of 1:150 was obtained in pigeon No. 99, third group, Antigen IX, the specificity was not high, the reaction occurring at 1:20 with both rat and mouse serum. Since TROMMSDORF and UHLENHUTH and WEIDANZ have shown that serologically the rat and mouse are widely separated, a precipitin which reacts equally for each cannot be considered acceptable for the study of species differences within the order Rodentia. Even a lower grade of specificity is observed in the case of pigeons No. 95 and No. 97, second group. This is logical from the commonly observed fact that the specificity of antibody reactions may diminish with too prolonged period of treatment of the animal being immunized.

Twenty-one birds were immunized with antigen No. X–A, B, C, D, E, human serum from the five races. This series was a control originally designed as a corollary to the serological study of rodent species relationship. It was thought that in the event that precipitating antisera from pigeons could differentiate the four strains of mice being studied, it would be interesting to demonstrate simultaneously the possibility of similarly differentiating or not differentiating the races of man. If a difference had

been observed it might have been indication of more, or less, serological, racial distinction correlated with higher levels of phylogeny.

All of the birds were immunized by three injections, 1, 2 and 3 cc, given at three day intervals, and eleven birds gave positive sera, reacting at levels up to 1:100 dilution of antigen. All sera were completely non-specific in cross tests against serum from the four other human races, but showed homologous specificity when titrated against rodent serum.

It is notable throughout that while several pigeons were always lost during the course of immunization to mouse or rat serum, human or rabbit serum never gave toxic or other reactions when used as an antigen.

Antigens No. XI, XII, and XIII, sera respectively from *Rattus rattus norvegicus*, *Rattus rattus tectorum* and *Rattus rattus rattus*, were used to immunize fourteen pigeons with the object of studying possible reciprocal relationships of value. A preliminary series of inoculations demonstrated that more than 1.0 cc of rat serum was highly toxic for pigeons. Accordingly a mode of injection was adopted which gave little possibility of overwhelming the birds and which still approached the lethal dose sufficiently to insure highest antibody production. Injections were given every other day in amounts increased 0.1 cc each injection and ranging from 0.1 to 0.8 cc. Of fourteen birds so treated, five gave positive sera, reacting up to 1:20 dilution of antigen. These sera were entirely non-specific within the rodent group.

Antigen No. XIV, an extract of dried muscle tissue from Japanese waltzer, elicited no precipitin in five pigeons, when administered four days a week for two weeks, at dosages increased from 0.5 cc to 3.0 cc.

Fresh horse serum, antigen No. XV, injected into five pigeons, in amounts of 1, 2 and 3 cc, at three-day intervals, gave low-titer, non-specific precipitin in the sera of two of the five pigeons treated. Ten samples of normal pigeon serum were found to be free from natural precipitins for horse serum. The pre-immunization sample of serum from pigeon No. 157 in this series showed no precipitin for horse serum, but the treatment induced a precipitin which was remarkable in that it reacted identically up to a dilution of 1:20 with human, mouse, and horse serum. If more of this antiserum had been available we should have liked to determine the degree of ubiquity demonstrable for the non-specific reaction shown by this precipitin.

Work with the Antigen "OT" consisted in the sterile, operative transfer of parenchymatous tissues from *Mus musculus* to the peritoneal cavity of five pigeons. Whole or chopped liver, spleen, kidney and testis were

transferred. All incisions in the pigeon healed by first intention. It was hoped that slow resorption from the peritoneal surface might elicit a specific precipitin reaction in pigeons where no other method had been successful. The tissues transferred were lysed and either absorbed or encapsulated after two months, but the pigeons never developed any precipitin in their serum.

Related study of anaphylactic shock in pigeons sensitized to mouse protein

This study was undertaken on the basis of the theory that precipitin production is closely related to the development of the anaphylactic state. GAHRINGER has shown that pigeons may be sensitized to dog serum and made to react vigorously in anaphylactic shock. An interpretation of ZINSSER's "unitarian" theory of antibody production suggests that pigeons failing to produce specific and high titer precipitin reactions should also fail to develop the anaphylactic state.

The technique best adapted to the production of anaphylactic reactions in pigeons has been worked out by GAHRINGER. It consists in the preliminary dose of 0.25 cc of the serum to be used for sensitization and the subsequent administration after a two week interval of 0.5 cc of the homologous antigen. Both injections are intravenous. One hundred pigeons were sensitized by this method to the serum of *Mus musculus*. After two weeks the birds were divided into eight groups for the administration of the shock dose.

A first group of thirty was given the homologous antigen. Fifteen received 0.5 cc and their reaction was very slight being confined to transient dyspnoea and slight staggering. All symptoms were gone ten minutes after the injection and the birds were apparently perfectly normal. Five were given 2 cc of the mouse, homologous serum, and of these, two showed a moderate reaction. Immediately after the critical injection these two birds were relaxed. After a short interval they began to struggle and developed slight polypnoea. The posture was awkward with feet wide apart and the head down. There was drooling and relaxation of the sphincter. When placed in the cage after ten minutes they could fly and recovered rapidly so that they appeared to be normal in twenty minutes after their injection. The other three of this group of five gave a slight reaction, not more vigorous than the ones induced by the standard 0.5 cc injection.

During the two weeks incubation between the first and second doses the remaining ten of the first thirty had been kept outdoors at low temperatures. Five of these received 0.5 cc and five received 2 cc of the homol-

ogous antigen as a shock dose. No reaction occurred, even with the larger dose. The work of FRIEDBERGER and SEIDENBERG indicates that elevated metabolism necessary to keep animals warm at low surrounding temperatures leads to destruction of antigen in such a manner that the anaphylactic state is not induced. Such a conclusion may be borne out by the fact that here we find pigeons kept at lower temperatures absolutely refractory to a large shock dose of the homologous antigen. In addition the contrast demonstrated marks the reactions obtained in the birds kept inside as one of definite although slight anaphylaxis. At this point we might note that the general failure of high antibody production observed in this work may have been due entirely to the high temperature and accompanying high metabolic rate normally present in pigeons.

Of the hundred pigeons given a sensitizing dose of serum of the *Mus musculus* the remaining seventy were divided into seven groups of ten each. The purpose of this division was the determination of the specificity of the reactions observed with the homologous antigen. These groups received 0.5 cc of the following heterologous antigens respectively: Serum of Japanese waltzing mouse, *Rattus rattus* serum, rabbit serum, guinea pig serum, human serum, chicken serum and physiological salt solution. In no instances was the reaction either greater or less than the one observed in the case of the first fifteen birds which received 0.5 cc of the homologous antigen.

A detailed record of the reactions is not significant as the result may be summarized by the statement that the reactions were only slight even under the method of sensitization determined to be ideal by GAHRINGER. The reaction was equally marked when the second injection was physiological salt solution. This result with rodent protein as an antigen is the opposite of that obtained by GAHRINGER using dog serum as an antigen. It is worthy of note here that TROMMSDORF found it impossible to produce in either rats or mice anaphylactic phenomena using chicken protein as an antigen. That the taxonometric relation of the mouse and dog might account for the differences observed in the shock phenomena as seen by GAHRINGER and by us is problematical.

The presence of a precipitating antibody in serum may or may not bear a relationship quantitatively to the presence of any other antibody but we must consider it an interesting matter of record that the anaphylactic reaction of these pigeons was grossly identical with the precipitin reaction in that both were slight and both were non-specific. (Non-specific is meant in this instance to refer to the differentiation of the rodent proteins under consideration.)

Correlation

It is of possible wide interest in this work to study the general nature of antibody production in birds. Of particular note is the work of GUYER and SMITH in the production of lens antibodies in birds. HUXLEY-CARR SAUNDERS and FINLAY have approached this work from different angles but possibly the most difficult part of the technique employed by GUYER is that of estimation of the cytolysin content of serum from immunized birds. He used the precipitin reaction as a gauge of the cytolysin content of serum from immunized birds. The assumption that there is a quantitative proportional development of one antibody with that of another is a very great one; further, there is considerable evidence that antibody production in birds is not as facile as it is in mammals. Considering the fact that with identical amounts of antigen no two animals will ever react with the same titer of antibody formation it is difficult to establish in the absence of experimentation that cytolysin formation is parallel to the precipitin formation in birds.

Considering the technical difficulties found in the determination of precipitin content of pigeon serum, the method used by GUYER, in which the necessity for the presence of a definite precipitate in addition to clouding of the antigen-antiserum mixture was not stressed, may be insufficient to establish the existence of a definite precipitating antibody in fowl serum. In the absence of very perfect titration of precipitin content any correlated estimation of the cytolysin present seems impossible.

SOBOTKA and FRIEDLANDER have possibly given the only method of gauging other antibodies by the amount of precipitin present. They have shown that the precipitin titer of antipneumococcus sera is a gauge of the ability of that serum to protect against pneumonia.

SHERWOOD and DOWNS found it possible to induce high titer precipitin formation in the serum of roosters by the intraperitoneal injection of twenty cc of whole blood from the human, the sheep and the rabbit. This technique with mouse serum as an antigen and only four chickens as recipients did not give us antisera suitable for this study.

BERNARD PORTIS has described histological differences in the structure of the omentum in various species in correlation with the ability of that species to form antibodies. It would be very interesting to study the pigeon omentum with a view to the determination of the presence or absence of structures corresponding to the "Taches Laiteuses" (consisting chiefly of clasmatocytes) found in rabbit omentum. If these tissues may be accepted as having a definite role in antibody production following the intraperi-

toneal injection of antigen, then in the pigeon we might anticipate that they would be very scant, a condition analogous to that of the guinea pig. Correspondingly, the guinea pig is known to be unsatisfactory as an antibody producer.

BOYDEN, in a review of the subject of the precipitin reaction in relation to the study of animal relationships, has pointed out that "the titer of an antiserum is directly proportional to the concentration of antigen within its limits of reaction," and considers, therefore, that total nitrogen determinations should be made on all antigens to insure comparable results. This problem was obviated in this work by the use of large numbers of repeat antigen dilutions. In the work spoken of particularly by Doctor BOYDEN a sample of serum obtained by bleeding a single dog should, of course, be subjected to total nitrogen determination and diluted as antigen accordingly. In work with mice a serum to be so diluted might be obtained from a composite sample of blood of many mice thereby eliminating the factor of individual, hourly, daily or other variabilities in the total nitrogen content. In addition antigen dilutions, to establish the identity of the strains of mice studied, were made from many, many individuals with many repetitions on individuals with uniform results so that end-point determinations are relatively significant regardless of what the total nitrogen of serum might have been at the beginning. It is perfectly possible, however, that every consistent and specific serological difference observed in this work may have a correlated difference in total nitrogen content of antigen, but if such were the case it might also be assumed that the whole phylogenetic series as worked out by NUTTALL is also based upon total nitrogen differences among the species. Considering the numbers of mice studied here, the factors of possible hourly or daily fluctuations of total nitrogen content of serum are of no significance.

A further comment is perhaps in place here concerning the whole matter of quantitative precipitin studies. MANWARING and AZEVEDO have demonstrated "contradictory and paradoxical results" with quantitative precipitin reactions in the presence of complex protein mixtures. They consider these results to have a bearing on the study of biological relationships by the precipitin reaction whether done by the hematocrit or by end-point determinations. If it were true that end-point determinations as studied here were incapable of dependable evaluation, quantitative and specific precipitin reactions could not under any circumstances be used to study animal relationships. The point of difference in the technique here is that under simpler conditions of protein mixtures than those employed

by MANWARING and AZEVEDO the specificity of precipitin reactions cannot be doubted.

This may be particularly emphasized by the recent work by D. C. CARPENTER.[3] Using the super-centrifuge he has been able to fractionate casein according to three molecular weights. This enormously sensitive method of differentiating closely allied proteins was found to be correlated with a specific serological response to those proteins in rabbits. The fact that the quantitative, end-point, precipitin test could, even in the presence of possible group reactions, differentiate and specifically identify the hitherto supposedly identical caseins, might well establish thc quantitative dependability of the test as applied to animal relationships.

Conclusions

1. By the methods employed in this work it is impossible to develop anti-rodent precipitating sera in pigeons to a titer sufficiently high to be of value in the study of blood relationships within the order Rodentia.

2. Failure of vigorous and specific precipitin production is found correlated directly with an uncertain and non-specific response to attempted development of the anaphylactic state.

II. PRECIPITATING ANTISERA BUILT UP IN RABBITS

Object

Since in the study of antisera from pigeons it was found impossible to distinguish serologically the four mice under consideration, it was necessary to choose some other animal as the antibody producer. At the suggestion of DOCTOR F. G. NOVY and following the work of UHLENHUTH and WEIDANZ, and R. TOMMSDORF, it was decided that an investigation of the precipitin response in rabbits to mouse protein should be undertaken.

Technique

Throughout the technique was similar to that employed in the pigeon study, with the following additions. Antisera were collected from rabbits by carotid bleeding. Where whole blood from the mouse was used as antigen it was quickly transferred from the cardiac puncture in the mouse to the rabbit vein and no precaution against coagulation was necessary. In the actual precipitin test the antigen dilution was made by the use of a Troemmer diluting pipette ordinarily employed for counting red cells.

[3] Address at WESTERN PENNSYLVANIA HOSPITAL, Institute of Pathology, October, 1929.

Distilled water was used as a diluent and the crystal clear resulting solution of whole blood brought up to the required concentration of sodium chloride and then further diluted with physiological salt solution as desired for end-point determinations. The original blood for this antigen preparation for the precipitin test is obtained from a drop of blood on the tip of the mouse tail. There is present in all final dilutions both a trace of fibrinogen and a trace of hemoglobin. These substances, being recognized as serologically identical in many species, may account for some of the group reactions observed.

Data

TABLE 1

End-point determinations with nine anti-Mus musculus rabbit sera.

ANTISERUM NUMBER	DATE	*Mus musculus* HOMOLOGOUS ANTIGEN	*Mus faeroensis*	JAPANESE WALTZER	*Mus bactrianus*	*Mus norvegicus*	HUMAN	CONTROLS
I	3/31/28	1/6,500	1/6500	1/4,000	1/4,000	1/200	—	—
II	4/13/28	1/10,000	1/10,000	1/4,500	1/4,500	1/180	—	—
III	4/16/28	1/3,000	1/3,000	1/1600	1/1,600	1/40	—	—
IV	4/26/28	1/3,000	1/3,000	1/2,000	1/2,000	1/100	—	—
V	5/27/28	1/5,500	1/5,000	1/4,000	1/4,000	1/100	—	—
VI	6/ 7/28	1/18,000	1/16,000	1/2,000	1/2,800	1/400	—	—
VII	6/25/28	1/12,000	1/10,000	1/3,600	1/3,600	1/200	—	—
VIII	8/ 1/28	1/50,000	1/50,000	1/8,000	1/6,000	1/600	—	—
IX	5/ 1/29	1/35,000	1/35,000	1/3,500	1/3,500	1/240	—	—

TABLE 2

End-point determinations with two anti-Japanese waltzer rabbit sera.

ANTISERUM NUMBER	DATE	JAPANESE WALTZER HOMOLOGOUS ANTIGEN	*Mus bactrianus*	*Mus faeroensis*	*Mus musculus*	*Mus norvegicus*	HUMAN	CONTROLS
I	3/10/29	1/2,000	1/2,000	1/1,600	1/1,600	1/10	—	—
II	4/ 4/29	1/5,000	1/4,800	1/2,000	1/2,000	1/100	—	—

TABLE 3

End-point determinations with anti-rat (Mus norvegicus) rabbit serum.

ANTISERUM NUMBER	DATE	*Mus norvegicus* HOMOLOGOUS ANTIGEN	*Mus faeroensis*	*Mus musculus*	*Mus bactrianus*	JAPANESE WALTZER	HUMAN	CONTROLS
I	12/8/28	1/4,000	1/480	1/480	1/480	1/480	—	—

It was feared that rabbits might not be suitable animals for the production of rodent antisera, inasmuch as several investigators have considered that they might well contain natural precipitins for the other rodents. In an effort to eliminate this factor of error sixty-eight normal rabbits of assorted types and sexes were titrated against both *Mus musculus* and *Mus norvegicus* at dilutions of antigen ranging from 1:4 to 1:5,000 and were found to be consistently negative. In addition, all experimental rabbits were tested for the presence of the precipitating antibody in their serum before the treatment was instituted and all final titrations contained controls with normal serum. All such tests were negative.

The technique of rabbit immunization was that which is standard for many types of antibody production. In general it was found that the daily injection of small amounts of the mouse serum or whole blood was a superior procedure to the administration of large doses at intervals of several days. The best antisera were obtained by the intravenous administration of 0.1 cc of the antigen for seven days, rest seven days, injections repeated again for seven days, rest seven days, injection for seven days, and bled out after from five to twelve days according to the results of preliminary titrations.

The rabbits lost weight during their treatment and only 25 percent of those immunized produced antisera of sufficient titer for use in this work.

All antisera were "ice-box ripened" for from three to six weeks before use and after this period were found to maintain their level of reactivity constant.

Discussion

The counter experiment involving the production of anti-Japanese waltzer rabbit serum was undertaken as a check upon the first work. As UHLENHUTH and WEIDANZ as well as TROMMSDORF found it possible to differentiate rats and mice by either anti-rat or anti-mouse sera, it was similarly found possible here to obtain the same biological grouping with either anti-*Mus musculus* or anti-Japanese waltzer sera. It was further hoped that the anti-*Mus norvegicus* serum might differentiate the four strains of mice being studied, but such was not the case with the one high titer anti-rat serum obtained. Intravenous injections of rat protein were so poorly tolerated by the rabbits that of six animals treated only one serum was recovered with sufficiently high titer to be of value in this work. No explanation is suggested for the failure of this anti-rat serum to be specific in its reaction with the four mouse proteins, beyond the fact that

in general it was found impossible to distinguish these four mouse proteins by any precipitin test at antigen dilutions as low as 1/500.

It is apparently anomalous that there is no specific ratio of the heterologous low titer end-points to the homologous high titer reactions. This may logically be accounted for by the fact that the greater the development of

FIGURE 2.—Graffic representation of average end-point determinations of the precipitin titer of nine anti-*Mus musculus* rabbit sera.

the titer of the precipitin reaction the more sensitive and specific it becomes.

Table 1 and its graphic representation in figure 2 show that there is a consistent difference between *Mus musculus* and *Mus faeroensis* on the one hand and *Mus bactrianus* and the Japanese waltzing mouse on the other. These data form the basis for more important conclusions later to

be drawn. Table 2 shows similar differences using rabbit sera sensitized to Japanese waltzing mouse blood. Table 3 shows that when the sensitization is carried on with rat blood the four types of mice are not distinguishable.

Conclusions

1. A clear cut serological identity is established for the two groups of mice, *Mus musculus*, *Mus faeroensis* and Japanese waltzer, *Mus bactrianus*.

2. This provides highly important evidence that (a) *Mus musculus* and *Mus bactrianus* are distinct species, (b) that the Japanese waltzing mouse is derived from *Mus bactrianus*, and (c) that *Mus faeroensis* should be classified as *Mus musculus faeroensis* rather than as a distinct species.

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GENETIC AND CYTOLOGICAL STUDIES ON FRAGARIA

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INTRODUCTION

The work of A. J. MANGELSDORF and EAST (1927), correcting the faulty conclusions of MILLARDET (1894), reestablished the genus Fragaria to a normal Mendelian status. The "faux hybrides" of MILLARDET were accounted for (1) by the occurrence of "maternals," due either to accidental selfing or to some form of pseudogamy and (2) by dominance in the F_1 of the characters found in the male parent. Certain features of the species crosses, including variability in F_1 populations of diploid×octoploid crosses, remained unexplained.

In the present work a cytological examination of these F_1 plants was undertaken. In addition, a number of diploid species, differing widely from *Fragaria vesca* both morphologically and in geographic distribution, became available for study. The various crossing relationships of these new types were investigated, together with the hybrid cytology of a number of the species crosses. An unusual amount of chromosome pairing was

[1] The work reported here and in the following paper was done in Forest Hills, Boston, Mass.

found in the F_1 of crosses between octoploid and diploid species, which seems to be partially correlated with temperature.

The basic chromosome number for the genus is seven. A list of the species utilized, grouped according to chromosome number, follows:

Diploid

Fragaria americana alba Porter
F. bracteata Heller
F. californica Chem. & Schlecht.
F. collina Ehrh.
F. maxima ? Received under this name.
F. mexicana Schlecht.
F. nilgerrensis Schlecht.
F. vesca L.
F. vesca rosea Rostr.
F. sp. (FPI 64856, from China).
F. sp. (429, from Hawaii).

Hexaploid

Fragaria elatior Ehrh.

Octoploid

Fragaria chiloensis Duchesne.
F. glauca Rydb.
F. platypetala Rydb.
F. virginiana Duchesne.
F. sp. (Friday Harbor, Washington).

Detailed descriptions of the species under investigation, with the exception of *F. sp.* (Friday Harbor, Washington) and *F. platypetala*, have been previously published by MANGELSDORF and EAST (1927) and by the writer (1927). With the exception of *F. nilgerrensis*, the diploid species are marked with the characteristics of a low chromosome number—rather thin leaves, petioles and scapes of small diameter, small flowers and fruits, and a rather light green foliage. Two diploid species, *F. collina* and *F. maxima*, show a striking similarity to *F. elatior*, a hexaploid type. *F. sp.* (FPI 64856, from China) differs from the *vescas* in having shorter leaflets that are darker green.

F. nilgerrensis, also native to China, has a leaf shape very similar to that of *F. chiloensis*. Its leaves are neither stiff nor glossy as those of *chiloensis* but are dull and pubescent. The scapes are short and the fruit white and of a distinctive fragrance.

F. elatior exhibits an increase in flower and fruit size and has stockier organs with a heavier pubescence than the diploid. The *chiloensis* forms of the octoploid group reach the highest degree of vigor, with thick petioles and stolons, and with flowers and fruits of immense size. The leaves become almost leathery and are very dark green. Such increase in size is less noticeable in the *virginiana* forms, although they are readily distinguishable from the diploid.

F. platypetala lies between *chiloensis* and *virginiana* in size and appearance. Its chief characteristic is its blue-green foliage. *F. sp.* from Friday Harbor, Washington, is much smaller than either type. The leaves are rather thin and glossy above and have a short petiole. The plant may be described as very procumbent with very short scapes, a characteristic which separates it from all of the other species under study.

METHODS

The technique employed in making the crosses is that described by MANGELSDORF and EAST (1927). Numerous emasculated flowers left unpollinated as checks died with no indication of setting seed. Root tips were examined cytologically from all of the species listed above and from a large proportion of the hybrid populations. Root tips were placed in Flemming's strong and weak fixatives and in a chrom-acetic-formaldehyde solution. Good results were obtained with all three. Sections were stained in Haidenhain's iron-alum-haematoxylin and iodine-crystal-violet. Buds were fixed in ALLEN's modification of Bouin's fluid and stained chiefly with haematoxylin. All drawings were made with the aid of the camera lucida, using (with the exception of figure 14) a 1.5 mm Spencer apochromatic objective and ×18 ocular.

DESCRIPTION OF CROSSES

Seven-chromosome species crossed inter se

The Hawaiian form, *F. sp.* 429, when crossed with *F. vesca* from Europe, gave vigorous, completely fertile progeny. In shape of leaves and fruit the plants are more like the Hawaiian parent. The fruit is red as in *vesca*. A cross between 429 and *F. americana alba* produced only white fruit in the F_1, indicating for both species the simple recessive white for fruit color. Reciprocal crosses between 429 and *F. bracteata* resulted in vigorous uniform progeny.

F. vesca rosea×*F. collina*

Sixteen plants were grown. They are vigorous, uniform and intermediate between the parents. The plants are only partially fertile however. The flowers are white instead of pink as expected, since the *vesca* parent was homozygous for the dominant factor for pink flower (*PP*).

The F_2 of the above cross shows a large amount of variation, especially in vigor; also in leaf shape and size, length of petiolule and in the type of serrations. Ten plants were grown. One died early because of lack of vigor. Four were classified as small. The rest range from moderately vigorous to extremely vigorous. Neither parental type was recovered. Pink appears in the corolla of certain F_2 individuals in a somewhat modified form which is neither the faint blush of the white-flowered *vesca* nor the pink venation of *vesca rosea*. It is a solid pink throughout the entire petal. Most of the flowers fail to set fruit.

Twenty-eight plants of the backcross of the F_1×*F. vesca* were grown. These plants also show considerable variation in vigor. Six plants were dwarfed, all dying before maturity. Seven were listed as small and have not as yet bloomed. The rest are vigorous, being more like the *vesca* parent.

Both F_2 and backcross plants vary in fertility as in other respects. Certain of the more vigorous plants appear to be almost completely fertile; others give no evidence of being able to bloom. Plants with the leaf shape of *collina* are among the less vigorous group.

F. vesca×*F. maxima*

The F_1 of this cross resembles that of the cross *F. vesca*×*F. collina;* the female parent is the same in each case and the male parents are very similar in appearance. The ten plants grown were vigorous, uniform and only partially fertile. The *vesca* parent was heterozygous for pink flower, but only white flowers appeared in the F_1 as in the preceding cross. Twelve pollinations were made to produce the reciprocal cross but all failed.

Four other crosses have given results similar to those obtained from the two species crosses just described. They are: *F. bracteata*×*F. collina*, *F. bracteata*×*F. maxima*, *F. sp.* 429×*F. collina* and *F. sp.* 429×*F. maxima*. A pistillate form of *bracteata* was used. The second cross shows some variation in leaf shape. The last cross is distinguished by especially large foliage, larger than any pure species or hybrid.

F. vesca×*F. sp.* FPI 64856

The seven plants grown were uniformly vigorous. There is some variability in leaf shape. The plants are partially fertile. Similar results were

obtained with *F. sp.* FPI 64856 both when *F. bracteata* and *F. sp.* 429 were used as the female parent. Both are partially fertile. Upon selfing, seeds of the former cross gave around fifty percent germination. A single maternal plant having white fruits that matured full sets of seed appeared in the latter cross. Its sibs vary greatly in vigor; those blooming proved to be nearly sterile.

F. vesca×*F. nilgerrensis*

Thirteen pollinations resulted in one small fruit with six seeds, four of which grew. The plants are uniform and of a peculiar type. Growth was very slow. At maturity the plants have many small leaves (less than one-third as large as those of either parent). The terminal leaflet, especially, tends to be triangular in shape with the apex at the base and the serrations mostly confined to the broad tip. The plants seldom produce runners. No flowers have appeared during the two years the plants have been in existence. Fifty seeds were obtained from the reciprocal cross, 32 of which germinated, all being exactly like the female parent.

F. bracteata×*F. nilgerrensis*

This cross was somewhat easier to make than the preceding one and the germination of seed was over 90 percent; but the plants proved to be of the same dwarfed type as before. Nineteen plants were grown, none of which has survived after two years. Seven attempts to produce the reciprocal cross failed.

F. sp. 429×*F. nilgerrensis*

Fifty seeds gave 22 plants, five of which are maternal. The remaining are true hybrids of the type described above, and show the greatest vigor of any plants of this type. The reciprocal cross consists of two plants from four seeds. Both are of the same dwarfed type.

F. nilgerrensis×*F. sp.* FPI 64856

Seven plants of the dwarfed type peculiar to crosses involving *F. nilgerrensis* were grown. A number of others died in the seedling stage. These plants were the weakest of the type.

F. collina×*F. maxima*

Two pollinations were made, fruit resulting in each case. Sixty-nine seeds gave ten plants (14.5 percent germination). Some variability was observed in both leaf shape and vigor, although none was far below normal in vigor.

Since the parents are morphologically similar, the F_1 differs from neither to any extent. The reciprocal cross also set fruit readily, and gave about the same percentage germination. The plants are uniform and vigorous. The plants of both crosses have been slow to come into bloom and for this reason fertility data can not now be included.

F. collina×*F. sp.* FPI 64856

This cross and its reciprocal were easily made. The germination was around 25 percent. The plants are fairly uniform and vigorous, and are partially fertile. The second generation of each varies considerably in vigor.

F. collina×*F. nilgerrensis*

Both this cross and its reciprocal set readily. The direct cross resulted in four plants, apparently true hybrids, uniform and fairly vigorous. Ten plants of the reciprocal cross were grown, three of which proved to be true hybrids. The remaining seven plants were maternal.

Crosses between 7- and 21-chromosome species

Attempts to cross the diploid Fragarias with the hexaploid species, *F. elatior*, fall into three groups: (1) complete failure to set fruit, (2) the production of seed that fails to germinate and (3) populations that are in every respect like the female parent. In two cases (*F. elatior*×*F. vesca* and *F. elatior*×*F. sp.* FPI 64856) all pollinations failed. The following crosses produced seeds but no plants: *F. collina*×*F. elatior* (139 seeds), *F. maxima* ×*F. elatior* (40 seeds), *F. sp.* FPI 64856×*F. elatior* (175 seeds) and *F. elatior*×*F. collina* (6 seeds). In addition, two crosses produced a single maternal each, *F. vesca*×*F. elatior* (406 seeds) and *F. elatior*×*F. maxima* (46 seeds). Since these single plants may have been due to accidental selfing, the crosses are placed in the group having defective seeds. This gives a total of 810 seeds that failed to germinate in crosses between the diploid and the hexaploid.

In the crosses to be described, only plants exactly like the female parent appeared. Since the differences between these species are relatively great, there is no difficulty in determining the sort of plants obtained.

F. sp. 429×*F. elatior*

Three pollinations resulted in 325 seeds, 18 of which germinated (5.7 percent). Eleven plants have bloomed. They had white fruit and were fully fertile.

F. nilgerrensis×*F. elatior*

Three out of five flowers set fruit, giving a total of 150 seeds, 18 percent of which germinated. The 28 plants all had the characteristic *nilgerrensis* foliage. Those fruiting had white berries.

F. elatior×*F. bracteata*

The six seeds obtained gave five plants, all of which were typical *elatior*.

F. elatior×*F. nilgerrensis*

Sixty-two seeds gave a germination of 11 percent. All plants were like the *elatior* parent.

Diploid×*octoploid crosses*

F. vesca×*F. sp.* (Friday Harbor)

This cross resulted in three maternals and three true hybrids of moderate vigor. The hybrids died before blooming.

F. nilgerrensis×*F. sp.* (Friday Harbor).

Three pollinations were made, all being successful. Only one of the 358 seeds germinated. This plant was a true hybrid of moderate vigor. It had bluish-green foliage and resembled the male parent in size and shape of leaf. It died before blooming.

In a cross of *nilgerrensis* with the commercial variety, Dunlap, used as male, four plants just like the mother were grown from 419 seeds. When *chiloensis* was used as the pollen parent 772 seeds were obtained, none of which grew. When *virginiana* pollen was used 594 seeds gave 0 percent germination.

The following attempts were unsuccessful when pollen from octoploid plants was used on *F. sp.* FPI 64856 (19 trials), *F. collina* (11 trials), and *F. maxima* (42 trials), making a total of 72 unsuccessful pollinations. All three species, however, give hybrids in the reciprocal cross.

F. californica×*F. chiloensis*

Thirteen seeds gave a population of five plants that show considerable variability. One plant died early. The remaining four vary as to leaf shape and thickness, length of petiolule and type of serrations.

F. vesca×*F. chiloensis*

Forty-six pollinations were made, a large number setting fruit. Only two families were raised from a total of 536 seeds. One family consisted of five

plants, quite uniform and vigorous, all true hybrids. Two of these hybrids have given progeny with *chiloensis* pollen, 16 additional pollinations failing. One of the F_1 plants thus successful in the backcross has given viable seed after open pollination in the greenhouse. Both the F_1 and later generations are very close to the *chiloensis* type. The other family includes five true hybrids plus nineteen plants that resembled only the mother. Of the hybrids, three were rather weak and died early. The other two are vigorous and show differences in leaf shape. One of these plants set fruit when growing out of doors. The seeds germinated very well, giving a large, uniform and vigorous population resembling *F. chiloensis*. Fruit resulted in two cases from pollinations of the F_1 with the diploid. The eight seeds obtained failed to germinate. Thirty-two additional pollinations were unsuccessful.

F. sp. 429×*F. chiloensis*

Seven plants were grown, six of which were obviously maternal; those blooming were fully fertile and had white fruit. The seventh plant, while similar to the mother is somewhat modified. It has bloomed profusely but shows reduced fertility, many of the flowers being entirely sterile. The pubescence of 429 is very slight above and practically absent below. This F_1 plant has increased pubescence above and somewhat more below than on the upper surface. The scapes also show an increase in pubescence. The leaflets tend to be narrower in proportion to length than do those of the female parent. The fruit are *red*.

F. vesca×*F. virginiana*

About one-third of the flowers pollinated set fruit. While the germination was low (from 5 to 10 percent) no lot of seed proved to be completely inviable. Ten families were raised, varying in size from one to fifteen plants. All progeny may be placed in three groups: (1) weak hybrids, 23 plants, (2) vigorous hybrids, 23 plants and (3) maternals, 2 plants. The family producing the two maternals consisted of these two plants only. While the female parent was a pistillate *vesca*, the progeny were both perfect flowered.

F. vesca×*F. glauca*

A single family of three plants was grown. It included one each of the above three types.

F. sp. 429×*F. virginiana*

The fourteen plants raised from this cross are all vigorous. While there is considerable variability as to leaf shape, on the whole the F_1 plants show a

greater likeness to the diploid parent than is true of any other cross of this type.

Octoploid×diploid crosses

F. chiloensis×F. bracteata

Three vigorous hybrids were obtained from three seeds. There is some variation in leaf shape. The plants closely resemble the *chiloensis* parent but show a lighter green foliage and a heavier pubescence on the upper surface of the leaves than does *chiloensis*.

F. chiloensis×F. collina

Thirty-two seeds gave twenty-eight plants (87.5 percent germination). Of this number four were weak, dying early; the rest appear to be vigorous hybrids. Chromosome counts made from root tips of two plants very similar to the mother showed thirty-five chromosomes. The influence of *collina* is usually evidenced by a narrowing of the leaves, which tend to be acute at the base. The leaves are dark green and thick as in *chiloensis*. The two plants that have flowered were completely sterile.

F. chiloensis×F. maxima

Forty-three plants were obtained (germination 42.5 percent). This cross is very similar to the preceding one. The influence of the male parent is less evident. No weak hybrids appeared. The five plants that have bloomed proved to be entirely sterile.

F. chiloensis×F. sp. FPI 64856

Forty percent of the fifteen seeds obtained germinated, giving six plants. One was very weak and died early. The others appear to be vigorous hybrids. One, very similar to the mother, bloomed without setting fruit.

F. chiloensis×F. nilgerrensis

Forty-eight seeds gave only one plant. It was a true hybrid and showed in general the type of dwarfness associated with hybrids of *nilgerrensis* parentage. In this case the leaves were thick and shiny. It died without blooming.

Seventeen *chiloensis* flowers were pollinated with *vesca* pollen and five with pollen from *F. sp.* 429, none of which set fruit.

F. virginiana×*F. collina*

Twenty-four plants were obtained from 31 seeds (77.4 percent germination). There is some variation in vigor but all apparently are true hybrids. Root tips were fixed from four vigorous plants. All showed thirty-five chromosomes. Eleven plants have flowered without setting fruit.

The following pollinations were unsuccessful, using *virginiana* flowers and pollen from the following sources: *F. bracteata* 9 trials, *F. vesca* 21 trials, *F. maxima* 26 trials, *F. sp.* FPI 64856 12 trials. Other octoploid types times the diploid gave 27 failures, making a total of 95 unsuccessful pollinations in this type of cross. *F. virginiana*×*F. nilgerrensis* gave 125 seeds but no plants.

Crosses between hexaploid and octoploid species

Nine attempts were made to cross *F. elatior* with *F. virginiana;* eleven attempts with *F. chiloensis*. All failed.

F. virginiana×*F. elatior*

This cross was made by Doctor A. J. MANGELSDORF. A pistillate form of *virginiana* was used. Twenty-five seeds gave twenty plants that show the dominant characteristics of *elatior*. The population is very uniform. The plants that have bloomed are completely sterile.

Crosses of octoploid species inter se

F. chiloensis×*F. platypetala*

Twenty-seven plants were grown, uniform except for sex. In leaf shape and thickness they resemble *chiloensis*. The color of the leaves is the bluish-green of platypetala. The plants are fully fertile.

F. virginiana×*F. sp.* (Friday Harbor)

These plants are also uniform except for sex. They are very similar to the male parent except that the leaves are larger and the plants more erect in habit of growth.

CYTOLOGICAL OBSERVATIONS

Crosses between diploid and octoploid

Pollen mother cells have been examined from the following crosses: *F. vesca*×*F. glauca, F. bracteata*×*F. virginiana, F. vesca*×*F. chiloensis, F. chiloensis*×*F. collina, F. chiloensis*×*F. maxima, F. chiloensis*×*F. sp.* FPI 64856 and *F. virginiana*×*F. collina*. The parent species differ widely both

in morphology and in chromosome numbers. These large differences are reflected in the irregular behavior at the reduction division of the F_1. The chromosomes are seldom well aligned on the metaphase plate at the first division. In fact, late diakinesis seems to merge into early anaphase in some cases and a true first metaphase stage is absent. Multipolar spindles sometimes occur at second metaphase. The resulting cells range from two to six or seven in number. The pollen grains vary greatly in size, few if any being normal.

Both the diploid times octoploid and the reciprocal show the same general type of behavior at meiosis. All F_1 plants tend to have complete pairing among the chromosomes. Since the somatic number is 35, complete pairing would give 17 or 18 bodies on the first metaphase plate. Because of certain irregularities, this number varies from 12 to 24. Such irregularities are of two types, (1) the association of more than two chromosomes and (2) failure of chromosomes to effect any association. Both types are found to occur together. Usually a plant shows more instances of one type than of the other. Examples of each may be found in table 1 where first metaphase counts are given for pollen mother cells from single buds of two plants.

TABLE 1

COUNTS AT I M	12	13	14	15	16	17	18	19	20	21	22	23	24
Plant 219–3	0	0	0	0	0	0	24	14	17	13	6	2	1
Plant 409–1	7	16	28	39	47	21	8	3	2	0	0	0	0

The two plants have a similar parentage: 219–3 is from the cross *F. vesca*×*F. glauca* and 409–1 is from the cross *F. vesca*×*F. virginiana*. Where first metaphase counts of from 12 to 17 are found, association of more than two chromosomes must have taken place. Counts of from 18 to 20 show association among the non-homologous chromosomes of a single genom, since on a polyploid basis a haploid group of seven has been received from the female parent and four groups or genoms from the male. If the seven chromosomes from the female mate with seven from the male twenty-one chromosomes remain. If then two groups of seven from the *glauca* parent unite there still is left a group of seven that must be considered as consisting of non-homologous chromosomes. Counts of from 21 to 28 indicate association of chromosomes between the genoms of the octoploid parent.

It will be seen that in the case of plant 219–3 groups of more than two chromosomes rarely occur in this bud if at all. Eighteen, the class of complete pairing (17 bivalents plus one univalent) is also the one of highest

frequency—31 percent of the plates examined. In 18 percent of the plates four of the non-homologous chromosomes form two groups; in 22 percent, two non-homologs have united; in 28.5 percent, no pairing is evident in the odd genom.

In the second case (plant 409–1), there is a strong tendency for further association. Sixteen bodies are found at first metaphase most frequently. This means, in most cases, that two bivalents have associated to give a group of four. In some instances, however, two groups of four are found. Such groups of four usually can be readily distinguished.

Complete pairing, with the two types of variation described above, can be used as a basis for interpreting first metaphase counts (see table 2).

TABLE 2

*$11_{II} + 13_{I}$	would give 24 bodies at I M
$12_{II} + 11_{I}$	would give 23 bodies at I M
$13_{II} + 9_{I}$	would give 22 bodies at I M
$14_{II} + 7_{I}$	would give 21 bodies at I M
$15_{II} + 5_{I}$	would give 20 bodies at I M
$16_{II} + 3_{I}$	would give 19 bodies at I M
$17_{II} + 1_{I}$	would give 18 bodies at I M
$16_{II} + 1_{III}$	would give 17 bodies at I M
$14_{II} + 1_{III} + 1_{IV}$	would give 16 bodies at I M
$12_{II} + 1_{III} + 2_{IV}$	would give 15 bodies at I M
$10_{II} + 1_{III} + 3_{IV}$	would give 14 bodies at I M
$8_{II} + 1_{III} + 4_{IV}$	would give 13 bodies at I M
$6_{II} + 1_{III} + 5_{IV}$	would give 12 bodies at I M
The following additional groupings involving both types of variations have been found:	
$1_{IV} + 15_{II} + 1_{I}$	to give 17 bodies at I M
$2_{IV} + 12_{II} + 3_{I}$	to give 17 bodies at I M
$3_{IV} + 10_{II} + 3_{I}$	to give 16 bodies at I M
$4_{IV} + 8_{II} + 3_{I}$	to give 15 bodies at I M

* The number of single chromosomes in the group is indicated by the Roman numeral.

A few of the above combinations are illustrated in plate 1. Figure 1 was drawn from a late diakinesis stage. Three tetrasomes may be seen on the upper left; a trisome is just below the center, and ten bivalent chromosomes are scattered on the periphery. A metaphase plate showing sixteen bivalents and one trivalent may be seen in figure 2. The trisome is at the bottom. In figure 3 two tetravalents can be easily distinguished. One trivalent and 12 bivalents complete the number. The plate in figure 4 contains four tetravalents that are readily apparent. It is somewhat difficult, however, to identify with certainty the three univalents among the eight bivalents. Figure 5 illustrates a late metaphase. Five univalent chromosomes

LEGEND FOR PLATE 1

All figures are from pollen mother cells.

FIGURE 1.—Pistillate *F. virginiana*×*F. collina;* late diakinesis showing complete pairing with secondary association.

FIGURE 2.—*F. chiloensis*×*F. maxima;* first metaphase with complete pairing.

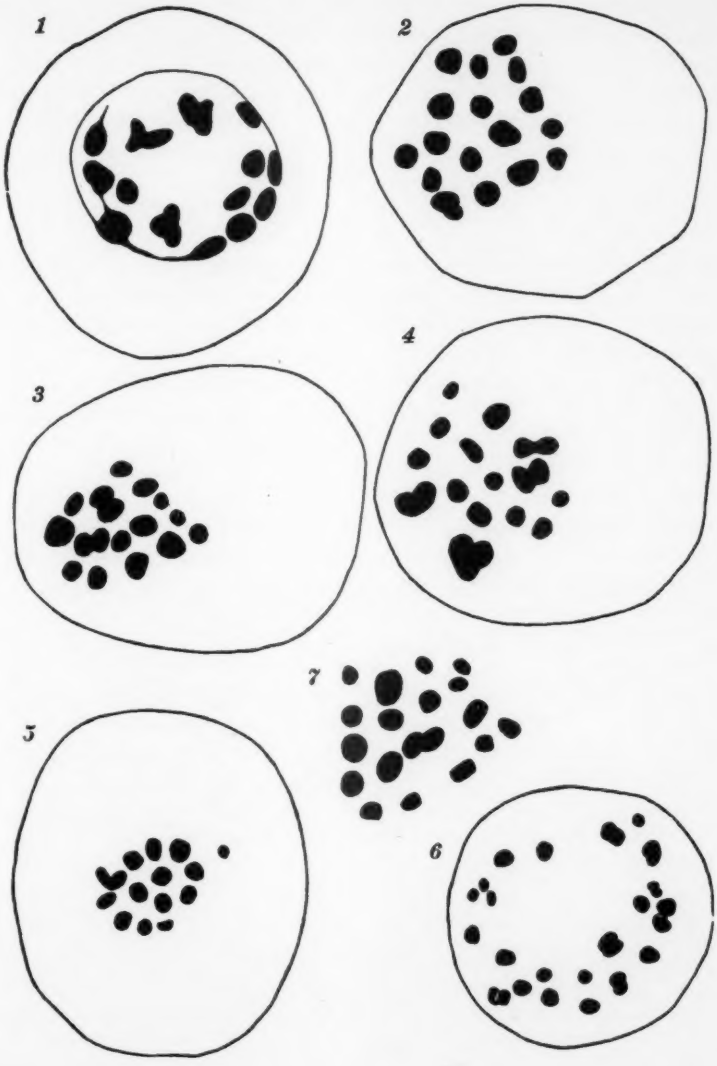
FIGURE 3.—*F. chiloensis*×*F. maxima;* first metaphase with two tetrasomes.

FIGURE 4.—*F. chiloensis*×*F. maxima;* four tetrasomes at first metaphase.

FIGURE 5.—*F. vesca*×*F. virginiana;* late metaphase.

FIGURE 6.—*F. chiloensis*×*F. maxima;* first metaphase, 11 bivalent+13 univalent chromosomes.

FIGURE 7.—*F. chiloensis*×*F. maxima;* first metaphase with one trivalent, 14 bivalent and 3 univalent chromosomes.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are already at one pole and three at the other. These are not included in the drawing. One trivalent (at left), two univalents (below and at right), and eleven bivalents remain on the plate. In figure 6 the chromosomes form a circular hollow "plate" which is seen occasionally. Eleven bivalents and thirteen univalents give a total of 24 metaphase chromosomes. Most of the bivalents can be distinguished without difficulty; one, at the lower left, is partially covered by a univalent. Figure 7 contains one trivalent, fourteen bivalent and three univalent chromosomes.

Temperature data

An attempt was made to correlate temperature with the variation in pairing exhibited in the above species hybrids. The dates of all fixations having been recorded, weather conditions for this vicinity for those days on which buds had been taken were obtained from the United States Weather Bureau in Boston. All fixations were made from plants in the greenhouse from 8 until 11 in the morning. Since all the days on which fixations had been made were cloudy, the sun did not greatly influence the temperature of the plants. Fires when present were banked at night so that the greenhouse cooled down to around 45° by early morning. It so happened that all fixations were made on days on which the temperature was either close to freezing or above. With such conditions it is believed that differences in out of door temperatures, while not the same, were fairly accurately reflected in greenhouse temperatures. Results are given in table 3.

TABLE 3

PLANT	CROSS	DATE FIXATION	WEATHER	OUTSIDE (F) TEMPERATURE	TYPE PAIRING
219– 3	*F. vesca*×*F. glauca*	12–16–27	Rain	36°	I
321– 4	*F. bracteata*×*F. virginiana*	6– 5–28	Cloudy	55°–56°	Intermediate
330– 2	*F. vesca*×*F. virginiana*	6– 5–28	Cloudy	55°–56°	Intermediate
409– 1	*F. vesca*×*F. virginiana*	6– 9–28	Cloudy	61°–64°	II
335– 3	*F. vesca*×*F. virginiana*	6– 9–28	Cloudy	61°–64°	II
321– 6	*F. bracteata*×*F. virginiana*	6–28–28	Partially cloudy	68°–74°	II
587– 7	*F. vesca*×*F. virginiana*	2–11–29	Cloudy	24°–27°	I
563– 9	*F. chiloensis*×*F. collina*	2–26–29	Cloudy	29°–33°	I
587– 6	*F. vesca*×*F. virginiana*	4– 1–29	Cloudy	42°–43°	Intermediate*
565–12	*F. chiloensis*×*F. maxima*	4– 1–29	Cloudy	42°–43°	Intermediate*
564– 4	*F. virginiana*×*F. collina*	4– 9–29	Cloudy	47°–50°	Intermediate
564–10	*F. virginiana*×*F. collina*	4– 9–29	Cloudy	47°–50°	Intermediate
565–12	*F. chiloensis*×*F. maxima*	4–19–29	Cloudy to 10:30	42°–47°	Intermediate*
553– 1	*F. vesca*×*F. chiloensis*	4–20–29	Partially cloudy	48°–54°	II

* Very little secondary pairing.

Type I is illustrated in table 1 by plant 219–3, in which no secondary pairing was observed and an appreciable number of single chromosomes was present. Type II is that of plant 409–1 in which there is a large amount of secondary pairing with only a very few univalents present.

Octoploid×hexaploid crosses

The pollen mother cells of the *F. virginiana×F. elatior* cross show a large amount of irregularity during meiosis. Cells at various stages of division are found side by side. The number of chromosomes on the plate at first metaphase varies greatly. There seems to be a small and irregular amount of pairing. The probable arrangement of chromosomes could not be made out. The chromosomes are greatly scattered at first anaphase. The most conspicuous feature is the presence of a plasmodium, illustrated in figure 14, plate 2, similar to that described for the Zea triploid by McCLINTOCK (1929). The number of cells coalescing ranges from two to a very large number, including all of the cells for some distance in the locule. The nuclei, most of which were in prophase though some were in first metaphase, had preserved their identity. The cytological findings are consistent with the complete sterility[2] exhibited in the F_1 plants. Figure 13, plate 2 shows 28 chromosomes at first metaphase, the nearest approach to "regularity" observed.

Somatic chromosome numbers

Root tips were fixed from a large number of the hybrids. Plants in every respect like the female parent have the same number of chromosomes as that parent. Most of the plants that gave evidence of a hybrid origin showed the combined gametic numbers of the parents. The dwarfed plants resulting from crosses between *F. nilgerrensis* and other diploid species had the diploid number of chromosomes. There were a few cases, however, where the expected chromosome number was not found.

In an F_2 of the cross *F. vesca×F. bracteata*, one of the plants (308–1) showed a slightly different type of foliage from its sibs. Two fixations were made of the root tips, an interval of several months intervening between the two. Upon examination, a single, comparatively large root tip was found on each slide that had 35 chromosomes in the metaphase plates. The five or six other root tips were very small and yielded no counts. Examination of the pollen mother cells from a single bud showed, as a rule, fourteen bivalents at first metaphase with a certain amount of irregularity in both

[2] WALDO and DARROW (1928) have found certain of their *virginiana-elatior* seedlings fertile. A cytological examination of the plants, however, is lacking.

LEGEND FOR PLATE 2

FIGURES 8–12.—Metaphase plates from root tip cells.

FIGURE 8.—*F. vesca rosea*×*F. glauca;* true hybrid with 14 chromosomes.

FIGURE 9.—*F. vesca*×*F. virginiana;* 56 chromosomes.

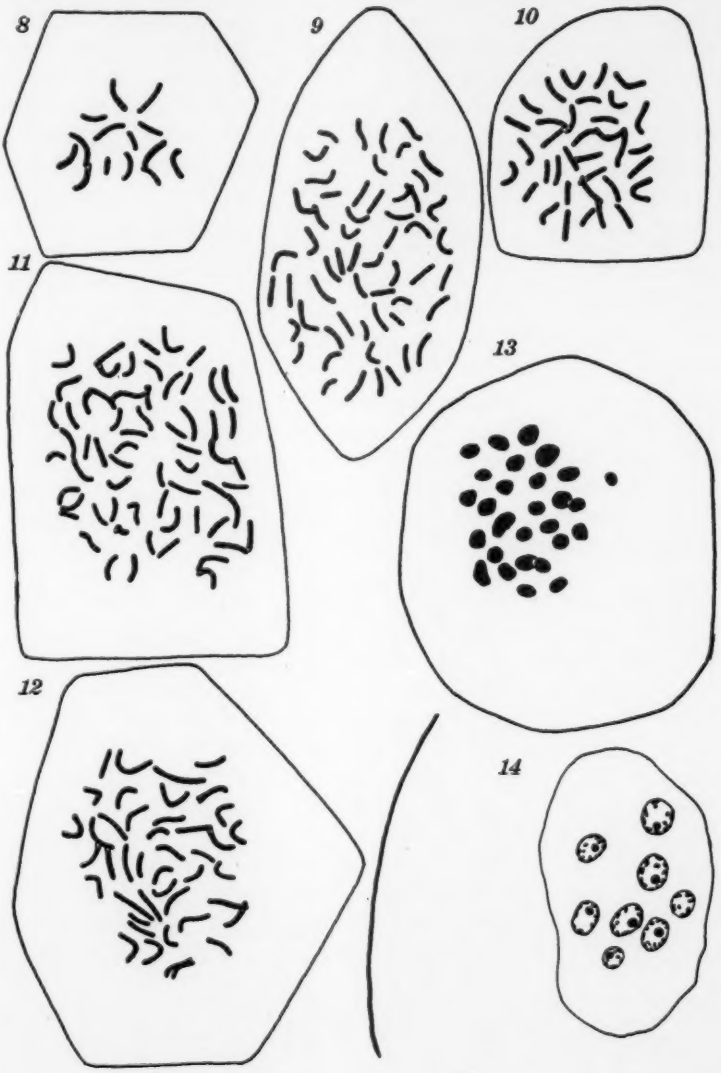
FIGURE 10.—F_2 of *F. vesca*×*F. bracteata;* 35 chromosomes.

FIGURE 11.—(*F. vesca*×*F. chiloensis*) F_1×*F. chiloensis;* 63 chromosomes.

FIGURE 12.—*F. vesca*×*F. virginiana;* 42 chromosomes.

FIGURE 13.—*F. grandiflora* var. Warfield×*F. elatior;* first metaphase plate with 28 bodies, possibly representing 21 univalent and 7 bivalent chromosomes.

FIGURE 14.—Pistillate *F. virginiana* No. 27×*F. elatior;* illustrating plasmodium; nuclei are all in diakinesis stage. ×440.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divisions. Metaphase plates from surrounding somatic tissue have 14 chromosomes. Flowers pollinated with *vesca* pollen gave a normal set of seeds, which produced a vigorous, uniform family. Five of these plants have 14 somatic chromosomes. Two have come into bloom and show a reduced fertility in the greenhouse. Unfortunately the original plant (308–1) failed to survive the drought during the summer of 1929. While the evidence is not entirely satisfactory, it indicates that plant 308–1 was a chromosomal chimera.

Seven plants in the 7×28 crosses have given unexpected somatic counts. The four crosses in which they appeared were reported by MANGELSDORF and EAST (1927).[3] Four of the plants from the cross *F. vesca rosea*×*F. glauca*, described on page 317 of their paper, were examined cytologically. Three are described as "weak dwarfs with miniature leaves." None have produced either runners or flowers. The other plant "is somewhat more vigorous; it produces many runners but it has never bloomed." The last plant finally flowered the following season, producing no fruit. All four plants have 14 chromosomes in the root tips (figure 8, plate 2).

The remaining three plants are from two families of the cross *F. vesca*× *F. virginiana*. Plants 324–3 and 349–2 were of the dwarf type described. Both had many small leaves with many fine serrations. One bore a few rather small flowers that were entirely sterile. Examination of the root tips showed 56 somatic chromosomes in each (figure 9, plate 2). The third plant also belonged to family 324. It was classed as "quite vigorous." The flowers were very small; the leaf-shape and serrations were *vesca*-like, but the leaf texture and color were *virginiana*-like. This plant had 42 chromosomes, illustrated in figure 12, plate 2. All but the most vigorous 14-chromosome plant have since died.

During February, 1929, a fruit set spontaneously in the greenhouse on a 35-chromosome plant of the cross *F. vesca*×*F. chiloensis*. The two plants resulting have given somatic counts of approximately 70 and 90 chromosomes respectively. A sister 35-chromosome plant in a backcross with *F. chiloensis* produced a plant with 63 somatic chromosomes (see figure 11, plate 2). The family consisted of 14 plants; unfortunately all but one failed to survive unfavorable conditions during the summer. An F_1 plant from another

[3] THOMPSON (1930, p. 416) is in error in supposing that these families consisted of "very weak seedlings, nearly all of which died in the seedling stage," and also that "LONGLEY (1926) showed that such seedlings were maternal and probably the result of parthenogenetic development." Three types were found: (1) like the female parent, (2) miniature dwarfs and (3) vigorous hybrids. LONGLEY (1926) reports only plants like the mother from the cross *Fragaria vesca*×*F. chiloensis*.

family of the same cross set two normal fruits out of doors. Counts made on the root tips of five plants of the uniform vigorous family resulting showed each to have the diploid number of chromosomes.

DISCUSSION

The earlier work on species hybridization at this laboratory, together with the results of MILLARDET (1894) and of SOLMS-LAUBACH (1907), has been concisely reviewed by EAST (1928). A more detailed report, previously referred to, has been given by MANGELSDORF and EAST (1926). References to earlier investigations, unless a citation is given, will refer to the work reported in this paper.

The diploid species under study may be divided into four groups on a basis of morphology, and crossing relationships, including degree of fertility of the F_1. The grouping thus determined follows:

I (a) *F. bracteata* Heller
F. californica Cham. & Schlecht.
(b) *F. vesca* L.
F. americana alba Porter
F. vesca rosea Rostrup.
F. mexicana Schlecht.
(c) *F. sp.* 429
II *F. collina* Ehrh.
F. maxima
III *F. nilgerrensis* Schlecht.
IV *F. sp.* FPI 64856

It had previously been shown that the species under (a) and (b) of group I are interfertile and as a rule produce uniform, vigorous progenies that are fully fertile. There seem to be some differences in vigor among the different F_1 populations, but they are comparatively slight. Morphologically the species belonging to the same subgroup are almost indistinguishable, aside from fruit and flower color. While members of the three different subgroups are readily distinguishable, all three have the same type of leaves, flowers and fruits. *F. sp.* 429 from Hawaii, the most vigorous of the group, retains the typical slender appearance characteristic of the *vescas*. In crosses with representatives of both subgroups (a) and (b) it has given vigorous, fertile progenies. ICHIJIMA (1926) reports normal behavior at meiosis for crosses among the species of the first two subgroups.

F. collina and *F. maxima* are perhaps less closely related than the species just discussed, but they still have much in common. They are very similar

in appearance, especially in leaf shape and in the type of serrations. *F. maxima* has the larger foliage. The texture of the leaves is somewhat stiffer than in the other diploid species. The long peduncles are characteristic of both. Reciprocal crosses are easily made. The F_1 are vigorous, although some variation in vigor was observed in the cross *F. collina*×*F. maxima*.

TABLE 4

Summary of crosses reported.

	F. bracteata	*F. vesca*	*F. sp.* 429	*F. collina*	*F. maxima*	*F. nilgerrensis*	*F. sp.* FPI 64856	*F. elatior*	*F. chiloensis*	*F. virginiana*
F. bracteata	—		v	v	vp	ds	vp		dvs	x
F. vesca		—	vf	vp	vp	ds	vp	n	mf dvs	mf dvs
F. sp. 429	v	vf	—	v	v	ds	vp	mf	mf	vs
F. collina				—	v	v	vp	n		x
F. maxima		x		v	—			n	x	x
F. nilgerrensis	x	mf	ds	mv	x	—	ds	mf	n	n
F. sp. FPI 64856		x		vp			—	n	x	x
F. elatior	mf	x		n	n	mf	x	—	x	x
F. chiloensis	vs	x	x	dvs	vs	ds	dvs	vs	—	
F. virginiana	x	x	x	vs	x	n	x	vs		—

Explanation: v—vigorous hybrids; d—dwarfed hybrids; m—maternal in appearance and behavior; f—fertile; p—partially fertile; s—sterile; n—nongerminating seed; x—failed to set fruit (at least five pollinations).

From the summary of all crosses in table 4 a comparison may be made of the behavior of the species in the different groups. Species used as female are listed on the left; those as pollen parent above. Crosses between the *vesca* group used as female and the *collina* group give vigorous progenies that are only partially fertile. The F_2 is exceedingly variable in vigor as is the backcross of the F_1 to *F. vesca*. The reciprocal cross was not fully tested out. In the case of *F. maxima*×*F. vesca* nine pollinations failed. When *F. collina* was pollinated with *F. vesca* three flowers gave every indication of setting fruit, but only a few seeds were obtained, none of which grew. The results indicate a more distant relationship between the two groups than among the species of either group. A preliminary study of the pollen mother cells of the F_1 shows a relatively slight amount of chromosome irregularity. An examination of the F_1 pollen stained in dilute iodine potassium iodide solution reveals very little good pollen, not over five percent. While many combinations of the two chromosome complements forming

the gametes are fully successful, it is equally evident that many are not, and certain of the corresponding chromosomes of the two species are by no means interchangeable. The gametes, containing the haploid number of chromosomes, would be expected to be very easily affected in development by an unusual chromosomal complement.

F. nilgerrensis varies widely in appearance from the other diploid species. It is likewise distinct in its genetic behavior. Where hybrids were obtained, in all cases except with *F. collina*, the plants were of an identical dwarf type, with many small leaves and without flowers. In crosses with *collina* the plants had a different leaf shape and were nearer the normal in vigor. When *nilgerrensis* was used as female, pollinations with *bracteata*, *vesca* and *maxima* were unsuccessful. The dwarf type of hybrids obtained seems to be a result of a peculiar complementary effect of the germ-plasm of the two parents. The lack of dwarfness in crosses with *collina* might be ascribed to the lack in that species of certain factors necessary to produce the dwarfing effect, or to the presence of other factors dominant to the influence from *nilgerrensis*. Either supposition sets off *collina* from the other groups. It is impossible to get an idea as to whether one or more than one factor is involved, since the F_1 plants are completely sterile.

A recent paper by HOLLINGSHEAD (1930) describes a somewhat similar situation in Crepis. Certain crosses between *C. tectorum* and *C. capillaris* gave only defective seedlings. Another similar cross gave half defective and half normal plants. It was found that a single factor in *C. tectorum* produced the defective F_1 when entering into the cross with *C. capillaris*. This effect was produced only in certain interspecific crosses; other such crosses and crosses within *C. tectorum* itself gave normal plants only. If this is the situation in crosses with *F. nilgerrensis*, all plants of this species tested must be homozygous for such a factor.

F. sp. FPI 64856, while similar to *vesca* in appearance, is nevertheless distinctive. It has rather dark green foliage which is thin in texture. It gives partially fertile hybrids with groups I and II, although attempts to cross with *vesca* pollen have failed completely. Hybrids with *F. nilgerrensis* gave the usual dwarf type.

In the previous work only four weak seedlings which died early resulted from over 600 seeds obtained from *F. vesca*×*F. elatior*. No flowers set in the reciprocal cross. The present results vary from the above in two respects, namely, in the absence of true hybrids from over 800 seeds obtained and in the appearance of plants exactly like the female parent. Since the similarity of the *collina* group to *F. elatior* is rather marked, it was thought that these species might show a closer genetic relationship than in the case

of the diploids of the *vesca* group. The results, however, were practically the same.

Results of crossing the *vesca* group with octoploid species for the pollen parent follow closely those already published. The germination was low; the hybrids show considerable variation in vigor, and certain populations include plants exclusively maternal in appearance. In contrast, the *collina* group and *F. sp.* FPI 64856 consistently did not set fruit with pollen from an octoploid source. *F. nilgerrensis* on the other hand yielded a single true hybrid of reduced vigor with *F. sp.* (Friday Harbor) from over 350 seeds. While it set fruit readily with *chiloensis* and *virginiana*, of the 1366 seeds obtained, none germinated.

Of the four diploid groups under consideration, the *vesca* type, which is the largest, has the widest distribution and likewise shows the greatest range of variability. Representatives are found in Europe, North and South America and the Hawaiian Islands. The *collina* group seems to be confined to Europe and the last two to Southeastern Asia. Species inhabiting the same general area might be expected to be more nearly related than species widely separated. A point to be considered in this connection is the method of reproduction. No data are at hand to determine the relative frequencies of the two modes of reproduction common in Fragaria. It would seem, however, that for plants growing under natural conditions asexual reproduction might dominate. If this is the case to a large extent, it would tend to slow up the evolutionary process in the species, both by reducing the number of new gametic combinations and by reducing the chance for the appearance of germinal mutations. Such behavior probably accounts for the similarity between the widely distributed species of the *vesca* type. While the Chinese species *F. nilgerrensis* does differ greatly from its geographically distant relatives, *vesca* and *collina*, it shows no closer relationship to the other Chinese Fragaria FPI 64856. In fact if vigor of the F_1 is to be used as the criterion, it is nearest the *collina* group.

MILLARDET (1894), RICHARDSON (1914), VAN FLEET (1919) and SCHIEMANN (1930) report the following results in octoploid×diploid crosses. MILLARDET obtained four "maternal" plants from the cross Docteur Nicaise, a cultivated variety×*F. vesca*. RICHARDSON, in a discussion of sex, reports for the cross *F. virginiana*×*F. vesca*, "a very few fertile hermaphrodites, sterile females, some apparently males and a large number of plants that did not flower." VAN FLEET crossed the commercial varieties, Chesapeake and Early Jersey Giant with a *vesca* type from South America. The F_1 was again pollinated with the same *vesca* pollen. The resulting plants were "everbearing," a character limited to the male parent. The ex-

cellent photographs do not disclose other *vesca* qualities. It is probable that the plants are of the "maternal" type often obtained in wide Fragaria crosses. Miss SCHIEMANN explained a variable population from the cross *F. grandiflora*×*F. hagenbachiana* as due to segregating characters of the very heterozygous mother plant.

All earlier attempts at this laboratory to obtain hybrids between the octoploid and the *vesca* group were unsuccessful. Later trials also resulted in failure, with the single exception of *F. chiloensis*×*F. bracteata* which yielded three plants. The *collina* group on the other hand gave large progenies when used as male with the octoploid. A single dwarf hybrid was obtained with the *nilgerrensis* pollen, and a small population with *F. sp.* FPI 64856.

In the case of the *chiloensis-bracteata* hybrids, the plants seemed to be closer to the *chiloensis* parent than in the reciprocal cross. With this single exception, crosses between octoploid and diploid species were successful in one direction but not in the other. It is possible of course that further trials might result in hybrids where failure is now recorded.

A consideration of meiosis in diploid-octoploid hybrids will be limited to a discussion of association at diakinesis and first metaphase, since the earlier stages of Fragaria are unsuited to detailed study and since the later stages are similar to those found in many wide species crosses. This material is rather exceptional in the amount of pairing observed. Pairing of chromosomes from the same parent has been found to occur in the cross *Crepis setosa* (4 haploid)×*C. biennis* (20 haploid), the F_1 showing 10 bivalents and 4 univalents (COLLINS and MANN 1923). When F_1 pollen was backcrossed on to the *biennis* parent, the resulting plants had 15 bivalents plus the univalents, giving evidence that *C. biennis* is an octoploid with the basic number of five (COLLINS, HOLLINGSHEAD and AVERY 1929).

The diploid-octoploid crosses in Fragaria result in plants having the pentaploid chromosome number. The pentaploid *Tulipa Clausiana* DC. was found by NEWTON and DARLINGTON (1929) to have 24 bivalents and 12 univalents part of the time; they also find groups of from three to five chromosomes. In the Fragaria material the pairing does not stop with association between homologs of four sets, but includes a part or all of the non-homologous chromosomes of the fifth group. There is no reason to suppose pairing between non-homologous chromosomes of four of the genoms. Polyploidy in Fragaria presumably has an evolutionary significance, the four gametic sets of the octoploid having an ultimate common origin. Corresponding chromosomes of each set then were originally homologous, whatever descriptive term may be most appropriate now.

The degree of chromosome identity cannot be determined by behavior at meiosis of the diploid-octoploid crosses, because pairing also takes place between chromosomes that can not be considered homologous. Pairing of the latter kind can not be distinguished in the cytological preparations of these hybrids. Similar behavior is found in the triploid, to be described later. Here non-homologous pairs can sometimes be identified. In the pentaploid hybrids association of two chromosomes at first metaphase is quite close. Degree of association may or may not be an indication of prophase relationships in this case.

It is supposed that secondary association between more than three chromosomes occurs at late prophase or early metaphase, and that association is between corresponding bivalents. It is looser than that found between two chromosomes, although considerable variation was observed. In the paper mentioned above, NEWTON and DARLINGTON (1929) say of the larger groups of associated chromosomes: "The forms of these bodies are for the most part very simple modifications of ordinary bivalents . . . ." They conclude that there is "great freedom of association" among the homologous chromosomes of the five genoms. In an earlier paper, DARLINGTON (1927) suggests that in hybridizing, chromosomes may be free to pair with "homologs" of another set from the same parent as well as from the other parent. It is not known whether chromosomes from the octoploid which pair with chromosomes from the diploid can also at times pair with "homologs" from the same gamete, but such is probably the case.

Crosses between *Aegilops cylindrica*×*Triticum vulgare* reported by H. and K. SAX (1924) gave 6 to 7 bivalents. This is interpreted as pairing between Aegilops and Triticum chromosomes. PERCIVAL (1926) and THOMPSON (1926) think there is no pairing between wheat and rye chromosomes. JENKINS (1929), studying the cross *Triticum turgidum*×*Aegilops speltoides*, however, finds from 4 to 10 bivalents in the F_1 with 7 by far the most frequent. He thinks that seven bivalents are formed by the mating of chromosomes from each parent, and that further pairing is between chromosomes from the wheat, since a small amount of pairing has been observed in a haploid wheat (GAINES and AASE 1926). The author suggests that, "It is also probable that factors other than affinity of individual chromosome pairs may affect the mating." JORGENSEN (1928) working on Solanum concludes that, "Other factors than the chromosomes themselves may influence the process of conjugation." CLAUSEN (1928) also warns that, "Cytological homology, however, is no assurance of genetic identity . . . ."

In regard to the variable amount of pairing in Triticum-Aegilops hybrids, KIHARA (1929) believes that an increase in temperature may in-

crease the amount of association. Fixations were made from the cross *Triticum dicoccoides*×*Aegilops ovata* on three separate days. Results for the last two days were very similar, while pairing was greatly reduced on the first day. Fixations from crosses between *Aegilops triuncialis*×*T. durum*, *T. polonicum* and *T. vulgare* were all made the same day. The first showed a mode of five pairs, the second a mode of six and the last had two and three bivalents in equal numbers. Temperatures were not given.

Since the fixations in Fragaria were not planned with the idea of observing the influence of temperature on the amount of pairing, and since differences in temperature are only approximate, the results can give but an indication of the possible relationship. In table 1 plant 219–3 represents the least amount of pairing found (called type I) and plant 409–1 presents an illustration of complete pairing with extensive secondary pairing (type II). The greenhouse temperature when the buds were taken was in each case higher than the temperature outside. There was, presumably, from 15°–20° F. difference between the two. Fixations were made in two cases in which the outside temperature was lower than in the fixation from 219–3 (see table 3, page 436). Both were of type I. One of these was an octoploid× diploid cross, involving species entirely different from the first two. In one instance (Plant 321–6), the outside temperature was higher than that for 409–1 and the weather was only partially cloudy. In this case no more association was observed than before. A bud from a cross similar to that of 409 was taken the same day and gave similar results. Buds from *F. vesca*×*F. virginiana* and *F. chiloensis*×*F. maxima* were fixed on the same day. Conditions of pairing were the same in both plants. In this case the amount of association was greatly reduced, corresponding to a lower outside temperature. In two instances buds were taken from the same plant on different days. When buds were fixed from plant 565–12, on both occasions the outside conditions were similar and the results were almost identical. The second fixation of the other case is not included in table 4. On February 27, 1930, a bud was fixed from plant 219–3 at about five in the afternoon, which had been sunny. The temperature inside the greenhouse at the time of fixation was 60° F., although it had been much warmer earlier in the afternoon. Upon examination little or no more association was found to occur than before. This is the only case in which the actual greenhouse temperature is recorded. It is assumed that the bud was about 10° warmer than the bud fixed on December 16, 1927. It may be that plant 219–3 is less responsive in this respect to an increase in temperature and that another ten degree increase would show an increase in association, although no influence of the higher temperature earlier in the afternoon was observed from this

fixation. In a bud taken April 1, a single plate gave counts of from 26 to 28 at first metaphase; this was the highest found.

While an increase in temperature seems to be correlated with an increase in the amount of association, it is not thought to be the only factor involved. The increase in association is of two kinds: the increased union of non-homologous chromosomes within a genom and the aggregation of pairs to give groups of four. It is interesting that pairing occurs between non-homologous chromosomes at lower temperatures than does secondary association between what are presumably two groups of related chromosomes. Similar behavior is found in the triploid.

Chromosome counts on the F_1 plants of crosses between diploid and octoploid species have established a rather remarkable series of polyploid chromosome numbers. Most of the plants, it is true, have 35 somatic chromosomes, the expected number, but several exceptional cases have been uncovered. Family 114 (*F. vesca rosea*×*F. glauca*) consisted of five plants. One died before a chromosome count was obtained. Three plants were too weak to produce runners or flowers, the fourth was moderately vigorous and bloomed. All had fourteen somatic chromosomes. Several things are to be noted in considering the origin of these plants. While the root tips were all small, with only a small amount of cell division, and the chromosomes were hard to stain properly, still the small number and size of the chromosomes made the counts certain. Duplicate fixations were made from three of the plants; all were alike. The morphological appearance of the plants together with conditions in the root tips (chromosomes of hybrids are in general much harder to stain properly than those of pure species) eliminates the possibility of a diploid number through accidental selfing or through any type of pseudogamy. This conclusion is further supported by the fact that the one plant blooming had white flowers. Since the *vesca* parent was homozygous for the factor for pink flower, plants of maternal origin should produce only flowers with pink petals. The fourteen chromosomes are probably made up of two complete sets, for abnormal chromosome combinations, as will be shown in a later paper, give very definite types unlike those found in family 114. Neither are the plants like any of the F_1's obtained in interspecific crosses among the diploid species in stock. There are three possibilities involving true fertilization by *glauca* pollen. (1) The production of seven-chromosome pollen by the octoploid parent, while conceivable on very rare occasions, can hardly explain the appearance of a family of four plants. (2) The elimination of twenty-one chromosomes at fertilization would explain the chromosome number, the hybrid condition, the variability in vigor, the white flower color and the

general resemblance of the F_1 plants to the *glauca* parent. Conditions favoring chromosome elimination in one ovule might be expected to be found in several or perhaps all in the same flower, which would result in a family of uniform chromosome number. The chromosomes eliminated might all come from the male parent or (3) the seven from the egg might be excluded with 14 from the male gamete, resulting in a form of merogony. It is difficult to choose between the last two possibilities. Variation in vigor within the family could be explained by assuming that a different set or combination from the octoploid parent remained to function in different eggs. The germination of the seeds was only 15 percent; perhaps some similar combinations may have been unsuccessful. Such a situation, occurring very infrequently, would be difficult to demonstrate cytologically.

A second case of the diploid chromosome number from the same type of cross was found in the cross *F. sp.* 429×*F. chiloensis*. The family consists of seven plants, six of which are entirely like the mother. The seventh is vigorous, but shows a likeness to the male parent and has proved to be partially sterile. It has 14 somatic chromosomes. The possibilities of origin just discussed apply equally here. The increased vigor might be accounted for by supposing that the set or sets from the octoploid were more compatible in this case than before.

HURST (1928) in a paper on polyploidy in the genus Rosa reports that, "frequently the somatic chromosomes of polyploids may be observed lying in groups of seven and in cases of serious physiological disturbance a whole septet of seven chromosomes may be cast out of the nucleus into the cytoplasm."

The hexaploid chromosome number is represented by a plant from the cross *F. vesca*×*F. virginiana*. The plant showed characteristics of both parents. Forty-two chromosomes were seen in root tip preparations. The plant proved to be entirely sterile. The simplest explanation of its origin seems to be a doubling of the chromosome complement of the female parent.

Two plants having the somatic chromosome number of the male parent were found. They were hybrids in appearance. One occurred in the same family as the 42-chromosome plant just discussed; it was weaker than its hexaploid sib. Two other plants of the same family had 35 somatic chromosomes. Both 56-chromosome plants had many small leaves. One bloomed, but buds in the right stage for a study of meiosis could not be obtained. They obviously were not the paternals of MILLARDET. If they represent cases of diploid merogony, one must ascribe a large influence to the maternal cytoplasm. In the case of diploid merogony in Nicotiana (CLAUSEN

and LAMMERTS 1929), the cross (*N. Tabacum*×*N. sylvestris*) F_1×*N. sylvestris* gave one plant out of 21 with 12 bivalents and exhibited only the characters of *sylvestris*, without apparent influence of the maternal cytoplasm.

Three other cases of abnormal chromosome behavior in Fragaria similar to the above but involving the tetraploid will be described in a later paper.

Fertility in the F_1 of diploid-octoploid crosses has been reported by MILLARDET (1894) in the cross *F. americana alba*×*F. chiloensis* and by RICHARDSON (1923). The latter found plants of the cross *F. vesca*×*F. virginiana* partially fertile. Twelve F_2 plants resembled the F_1 in foliage. The backcross with *virginiana* gave *virginiana*-like plants. The backcross with *vesca* failed. Five F_2 plants from open pollination outside were also obtained in the cross *F. virginiana*×*F. vesca;* only one has normal leaves. Only one or two seeds were produced by a flower.

In the present work fertility in this type of cross was found only in the cross *F. vesca*×*F. chiloensis*. All plants obtained from similar crosses had previously proved to be sterile. In attempts to backcross the F_1 to the diploid, the present results duplicated those of RICHARDSON. The only plant of the controlled backcross with *chiloensis* studied cytologically had 63 somatic chromosomes. If the functional eggs retain the somatic number (35), fertilization by a 28-chromosome pollen grain would produce the somatic number found.

Of the two plants examined which resulted from an open pollination in the greenhouse one had 70 somatic chromosomes. This plant has not as yet bloomed, but it would be expected to show a greatly increased fertility over the F_1. A number of similar cases have been reported: in Viola (J CLAUSEN 1924), in Raphanus-Brassica (KARPECHENKO 1924), in Nicotiana (CLAUSEN and GOODSPEED 1925), in Digitalis (BUXTON and NEWTON 1928) and in Crepis (COLLINS, HOLLINGSHEAD and AVERY 1929). KARPECHENKO (1928) finds that in the Raphanus-Brassica cross the F_1 was very polymorphous, as was found in the diploid-octoploid crosses of Fragaria. This was likewise true of the Crepis hybrids referred to above.

A sib to the above 70-chromosome plant has approximately 90 somatic chromosomes (probably the polyploid number 91). If the egg had the somatic number, the 91 chromosomes could be accounted for either by double fertilization with pollen from the octoploid, or more likely, since very little of such pollen could have been available, by the doubling of the chromosomes from the male gamete after fertilization.

In each of the above nine cases, the chromosome number has been found to be in the polyploid series and seems to involve complete sets or genoms;

thus the chromosomal balance has been preserved, but not the balance among genoms for a normal development. These groups of seven are evidently near enough alike to produce a viable plant in certain cases but not sufficiently so to form a normal plant. It is quite likely that some of the four distinct sets of the octoploid can no longer function normally in the diploid condition. It has been suggested by MANGELSDORF and EAST (1927) that the red fruited F_1 from the cross *F. americana alba* (white fruited) × *F. chiloensis* (red fruited) of MILLARDET might be explained by assuming chromosome elimination at fertilization, as in the case of family 114. If such an explanation is correct, one or more sets of the octoploid have remained relatively unchanged during the course of evolution, *chiloensis* being the result of an earlier cross. The *bracteata-virginiana* cross reported by MANGELSDORF and EAST (1927) resulted in 12 plants, ten of which were true hybrids. The other two were maternal except that they were pistillate, a condition previously unknown among diploids. If a set from the octoploid functions normally in the diploid condition, it might help to explain the appearance of numerous maternals in the diploid × octoploid crosses.

SUMMARY

1. The diploid species investigated have been divided into four groups on a basis of morphology and crossing relationships as follows:

I (a) *F. bracteata* Heller
F. californica Cham. & Schlecht.
(b) *F. vesca* L.
F. americana alba Porter
F. vesca rosea Rostrup.
(c) *F. Mexicana* Schlecht.
(c) *F. sp.* (429)
II *F. collina* Ehrh.
F. maxima
III *F. nilgerrensis* Schlecht.
IV *F. sp.* (FPI 64856)

Group I has been subdivided on a morphological basis.

2. Species within group I are interfertile. Groups I and II, I and IV, and II and IV give partially fertile hybrids.

3. Crosses between group III and groups I and IV give completely sterile hybrids that exhibit a peculiar type of dwarfness.

4. Hybrids between groups II and III, while reduced in vigor, are not of this dwarf type.

5. Crosses between the diploid×octoploid show considerable variability in vigor and morphological appearance, among plants of the same family.

6. The reciprocal octoploid-diploid cross shows less variation in vigor, but the plants vary in leaf shape, amount of pubescence, etc.

7. Certain F_1 plants of *F. vesca*×*F. chiloensis* proved to be partially fertile. A plant from the backcross to *chiloensis* having 63 somatic chromosomes, is probably the result of the union of an egg with the somatic number of the F_1 with a normal pollen grain from the octoploid. Two plants from an open pollination in the greenhouse have 70 and 91 chromosomes respectively. The first has twice the somatic number of the F_1, and the second is considered to be due to the union of an egg having the somatic number of chromosomes of the F_1 with a normal 28-chromosome pollen, the complement of which doubled after fertilization. An open pollination of the F_1 out of doors gave a large and uniform family. Five of these plants were found to possess the diploid chromosome number.

8. A cytological examination of buds from the diploid-octoploid crosses revealed pairing not only between chromosomes from both parents but also autosyndesis among the remaining sets of the octoploid parent. In many cases the counts at first metaphase indicated complete pairing, involving the non-homologous chromosomes of the fifth genom. In addition, secondary association was found to take place between disomes.

9. The amount of association showed considerable correlation with temperature conditions, indicating an increase in the amount of pairing with an increase in the temperature.

10. Plants from the diploid-octoploid crosses with unexpected chromosome numbers follow the polyploid series. One family of such a cross consisted of five true hybrids having the diploid chromosome number. Another family contained a plant with the hexaploid number and one with the octoploid number. A third family also had a plant with the paternal number of chromosomes. F_2 and backcross plants with various polyploid chromosome numbers have been mentioned above.

11. A cytological investigation of the buds of the F_1 of *F. virginiana*×*F. elatior* showed considerable irregularity. Plasmodial masses were observed in the pollen mother cells, that involved a variable number of cells.

12. Plants precisely like the mother appeared in all types of crosses. They were invariably completely fertile and many or most of such plants probably originated through some form of pseudogamy.

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A STUDY OF CERTAIN POLYPLOID AND ANEUPLOID FORMS IN FRAGARIA

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INTRODUCTION

The appearance in pedigree culture of a family of plants, the progeny of a species hybrid in which chromosome doubling had occurred, presented an opportunity for a study of the relationships of homologous chromosomes of two closely related species. It was also possible to compare the tetraploid

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form with the parental diploid types in out-crosses with a considerable number of distinct species, including representatives of the hexaploid and octoploid groups. Fertility with the parental diploid resulted in a series of types with aberrant chromosome numbers, which were distinct morphologically. The normal triploids resulting from diploid-tetraploid crosses were found to exhibit an unusual amount of chromosome pairing in reduction division. The genetic results of this type of meiosis have been followed through a later generation in crosses between triploid and diploid.

The technique of making the crosses is that described by MANGELSDORF and EAST (1927). No evidence of contamination, with the possible exception of accidental selfing, was noted in the controlled crosses. The cytological methods employed have been outlined in a companion paper, "Genetic and cytological studies on Fragaria," now in press.

The tetraploid family, and the stocks of pure species utilized, were kindly supplied by Doctor E. M. EAST, under whose direction the work was carried out. Thanks are also due Mr. A. MARSHAK who assisted in collecting the data on flower color, and Doctor P. C. MANGELSDORF for criticism of the manuscript.

A TETRAPLOID FAMILY

Origin

A family of seven plants having the tetraploid chromosome number originated as the selfed progeny of an F_1 plant (122–7) of the cross *F. bracteata* Heller (7)×*F. vesca rosea* Rost. (7) made by Doctor A. J. MANGELSDORF in 1924. A cytological examination of this F_1 individual, made by Mr. K. ICHIJIMA (1926) disclosed 14 bivalent chromosomes at the heterotypic metaphase. Root tip counts were not obtained from plant 122–7 which died after setting fruit, but its progeny, family 223, was found by him to have 28 somatic chromosomes.

The F_1 tetraploid Fragaria escaped detection during its lifetime. It is quite likely that this plant was in reality a chromosomal chimera, islands of tetraploid tissue having been observed in the root tips of diploids. Further, an F_2 of a cross similar to that producing the tetraploid, contained a plant that gave definite indications of being a chromosomal chimera. It is possible that the doubling, resulting in the production of family 223, took place in somatic tissue at some time during the development of the crown. There is direct cytological evidence that at least two additional flowers were in the tetraploid condition (ICHIJIMA 1926).

Reduction division in PMC

Meiosis in the F_2 tetraploid seems to be somewhat more irregular than was reported for the F_1 by ICHIJIMA (1926). While the prophases were not critically examined, there was no suggestion of irregularity through diakinesis, although four chromosomes were occasionally found to be associated at this stage. Whether there was any such significant association at first metaphase could not be determined. Usually 14 bivalent chromosomes could be distinguished. The genetic evidence indicates that association among the four homologous chromosomes to form bivalents is entirely fortuitous. That is, a chromosome from *vesca* seems to pair as readily with the corresponding one from *bracteata* as with its *vesca* homolog.

The movement of the chromosomes to the poles in the heterotypic division shows an occasional lagging pair (illustrated in figure 10, plate 1). Very rarely two lagging pairs may be seen. A large number of such cases must result in non-disjunction, as chromosomes are only rarely found in the cytoplasm until after interkinesis. Second anaphase is sometimes initiated by the early division of a single chromosome. Lagging chromosomes can likewise be seen at late anaphase, as well as an occasional chromosome in the cytoplasm. These latter do not form micronuclei. Tetrad formation is quite regular; the production of a fifth small cell is rare indeed. The amount of irregularity as described above varies somewhat among the seven F_2 plants and even for different buds from the same plant.

Meiosis in the F_3 plants follows closely that in the F_2. Some of the F_3 plants exhibit an increased amount of irregularity, especially at first anaphase with the chromosomes greatly scattered. A majority of these plants, however, show greater regularity at meiosis than does the F_2.

Inheritance in the tetraploid

It was shown by MANGELSDORF and EAST (1927) that pink in the veins of the petals is due to a single dominant factor (*P*). Plant 122–7 was heterozygous for pink flower color, since in *bracteata* the flower is white. All seven plants of the F_2 have pink flowers. In order to determine the mode of segregation in this tetraploid F_2 and to find out if these plants differ in genetic constitution, the plants of family 223 were crossed reciprocally with the white flowered *vesca*, *F. americana alba* (Porter), and were crossed with the white flowered parent, *F. bracteata* used as male. The plants were also allowed to self under conditions precluding contamination with foreign pollen. These results are to be found in table 1.

The backcross data for 223–5 represent the populations of two families of the cross *F. americana alba* × 223–5. A third family of similar origin has

given a segregation of 4 pink flowered to 18 white, which differs significantly from the 1:1 ratio of the other two families. It might be assumed that the F_1 plants of this cross fail to develop flower color when simplex for the dominant factor *P*. This would convert the expected 1:1 ratio to a 1:3 ratio. Such an assumption is hardly plausible since this would involve the appearance by mutation of an inhibiting factor in the *alba* parent, a plant which behaves normally (as judged by similar crosses), when crossed with

TABLE 1

Segregation of flower color.

BACK CROSS	FLOWER COLOR		DEV. FROM EXPECTED	RATIO	PROBABLE ERROR
	PINK	WHITE			
223–1	17	17	0	1:1	..
223–2	23	29	3	1:1	2.43
223–3	11	4	1.4	5:1	0.97
223–4	9	8	0.5	1:1	1.39
223–5	16	15	0.5	1:1	1.88
223–6	8	3	1.17	5:1	0.84
223–7	42	0	..	1:0	..
Selfed					
223–2	30	7	2.25	3:1	1.69
223–5	13	3	1.0	3:1	1.7
223–6	11	1	0.67	35:1	0.38
223–7	8	0	..	1:0	..

223–2, giving a population of 10 pink to 11 white. There is always the possibility of classifying a plant of the type *Ppp* as white-flowered, but this can hardly account for the preponderance of white-flowered plants in this particular family. At present no satisfactory explanation can be given, the data being disregarded in determining the genetic composition of plant 223–5. With this single exception the families all deviate from the expected by less than two times their probable error.

From these data it is assumed that plants 1, 2, 4 and 5 of family 223 are simplex for the gene *P;* plants 3 and 6 are duplex (*PPpp*) and plant 7 has either three or four dominant factors.

A doubling of chromosomes at some time after fertilization in the F_1 of a cross between two diploid species should result in the formation of tissue with two complete genoms from each of the species involved. Where the two parental species are each homozygous for simple allelomorphic factors, the F_1 which has doubled its chromosome number should contain four such factors, two identical with those of each parental species. This is the

situation postulated for the pink versus white color factors involved in the present tetraploid.

If the bivalents observed at diakinesis in the F_1 tetraploid are the result of chance association among the four homologs of each set, this plant should have given the following genotypic ratio upon selfing: 1 *PPPP*:8 *PPPp*:18 *PPpp*:8 *Ppppp*:1 *pppp*. If association was not fortuitous, but only between homologous chromosomes from the same species, only plants genotypically like the mother could appear upon selfing. A study of the seven F_2 plants gives the following distribution: 0 *PPPP*:1 *PPPp*:2 *PPpp*:4 *Ppppp*:0 *pppp*. While the observed segregation shows considerable deviation from expectation, the numbers involved are very small. The results seem to justify two conclusions, (1) pairing among the set of four homologs carrying the factors for flower color is a matter of chance, the excess of plants of the constitution *Ppppp* being evidence that homologs from the same species show no tendency toward preferential pairing, and (2) the F_1 plant was of the constitution *PPpp*. If the F_1 plant had the constitution *PPPp*, plants simplex for *P* could not segregate out by selfing. If its genotype were *Ppppp*, one would expect one-fourth of the F_2 to have white flowers, and no plants triplex for *P* could appear. Since such a plant was found and the F_2 was devoid of white-flowered plants, it is safe to assume that the F_1 was duplex for pink flower.

A comparison of the data from the backcrossed and selfed progenies shows them to be mutually compatible. Plants simplex for *P* should give a 1:1 ratio in the backcross and a 3:1 ratio when selfed. Both types of data were obtained from two tetraploid plants. Plants duplex for *P* should give a 5:1 ratio in the backcross and a 35:1 ratio when selfed. Plant 223–6 may be said to fulfil these requirements, although the small numbers involved conform to 3:1 and 15:1 ratios just about as well. However the 1:1 and 3:1 ratios are pretty well eliminated. Plants triplex for the dominant factor *P* could give no white flowered plants in either the backcrossed or selfed progenies. Plant 223–7, which yielded no white flowered progeny in either case, is placed in this category since the chance for the appearance of such a plant from a duplex parent is eight times that of a plant carrying four dominant factors.

The appearance of F_3 plants receiving complete chromosomal complements is much the same as that of the F_2. The more striking characters of the tetraploid, to be described later, seem to be due to the increase in chromosome number, and these characters appear again in the F_3. The smaller number of chromosomes of the triploid resulting from the backcrosses gives the segregation of slight differences from the tetraploid a bet-

ter chance to show up. These differences include small variations in the size and shape of the leaflets and serrations, length of petioles and petiolules, amount of pubescence, etc., largely due to the various combinations of *vesca* and *bracteata* chromosomes.

A tendency toward irregularity at meiosis seems to be typical of newly formed tetraploids. Thus GREGORY (1914) finds lagging chromosomes in his tetraploid Primulas; BELLING and BLAKESLEE (1924) report non-disjunction in about 25 percent of the pollen mother cells of the tetraploid Datura. The tetraploid raspberry variety, La France, shows an "irregular distribution of chromosomes, some of which lag on the spindle and are extruded from the daughter nuclei" (LONGLEY and DARROW 1924). GAIRDNER (1926) observed considerable lagging at first metaphase in a tetraploid Campanula. Others have found similar irregularities. Such behavior is often coexistent with a considerably reduced fertility. This is very noticeable in the tetraploid Fragaria. The fruits are much smaller than those of the diploid and exhibit a relatively large number of undeveloped achenes.

It would seem, from an examination of the pollen mother cells of family 223, that the opportunity for the formation of gametes lacking a chromosome would be greater than for the formation of gametes carrying an extra chromosome. Where such abnormalities were due to non-disjunction, an equal number of each might be expected, without taking a modification of viability into consideration. In addition to this, gametes lacking one or more chromosomes are formed where chromosomes are left in the cytoplasm. This point receives further attention in a later section.

The type of chromosome association in the Fragaria tetraploid as determined by the segregation of flower color follows MULLER'S expectation of random pairing in a tetraploid (MULLER 1914). It is also in agreement with segregation in the tetraploid Datura, where such adequate numbers have been obtained (BLAKESLEE, BELLING and FARNHAM 1923). The number of chromosomes associated at the heterotypic division seems to have no influence on their manner of segregation.

Crosses between 7- and 14- chromosome groups

Crosses of the tetraploid family 223 with the diploid proved to be relatively easy when the tetraploid was used as male, but much less so when used as female. Most of the resulting plants had 21, the triploid number of chromosomes. Since this is an unbalanced condition, the hybrids proved to be but slightly fertile. A number of crosses were made with *F. americana alba* Porter to determine the mode of segregation of flower color in the tetraploid. With the exception of those plants either lacking or having an

extra chromosome the populations were found to be relatively uniform and vigorous. They somewhat exceeded the diploid in vigor and could be distinguished by their coarser serrations, which were nearer the tetraploid type than the diploid. In addition to the above, crosses were made with the Hawaiian *vesca* (429), *F. bracteata* Heller and *F. nilgerrensis* Schlecht. used as female. Progeny of the first two crosses could scarcely be distinguished from that between *F. americana alba* and the tetraploid. The cross with *nilgerrensis* gave but four plants, three showing the same type of dwarfness found in crosses involving *nilgerrensis*, previously described (YARNELL 1931). The true hybrids were slightly more vigorous than the diploid hybrids with the same species, but none have bloomed, two having already died because of lack of vigor.

Crosses were successful with *F. americana alba*, *F. sp.* FPI 64856 and *F. collina* Ehrh. used as the pollen parent. Crosses with the first were identical with those of the reciprocal. Hybrids from crosses with the second species were quite variable. While one abnormal plant was found to have 20 somatic chromosomes, others just as abnormal had 21, the normal number for the cross. The cross with *collina* gave a uniform, vigorous population favoring the male parent. None set fruit either in the greenhouse or outside. Thirty-six additional pollinations of the type 4n×2n failed, 112 seeds from the same type of cross gave no plants.

F. elatior×*tetraploid and reciprocal*

Sixty-five seeds were obtained when *F. elatior* Ehrh. was pollinated with the tetraploid, but all failed to germinate. Sixteen plants were obtained from the reciprocal cross. They were uniform and more vigorous than either parent. The leaves were large with deep serrations like the tetraploid, while pubescence and flowers were more like those of *elatior*. The plants were found to be entirely sterile.

Octoploid and tetraploid crosses

Although sixty pollinations were made in an attempt to get a cross between the tetraploid *vesca* and the octoploid species, *F. chiloensis* L. and *F. virginiana* Duchesne, not a single flower set fruit. The reciprocal, (octoploid×tetraploid) was easy to make. On the whole the F_1 of this type of cross was quite variable. *F. chiloensis*×the tetraploid gave mostly weak plants plus a few vigorous plants very dissimilar in appearance. Five families with a total population of 67 plants of the cross *F. virginiana*×the tetraploid *vesca* were raised. Here again there was great morphological variability in the F_1 in regard to size of leaves and flowers, leaf shape and gen-

eral vigor. In no case did the ordinarily dominant pink flower color of the tetraploid appear.

This lack of color in the flowers of these F_1 plants may be explained as follows: Wide difference in the intensity of corolla color has been observed, which seems to be correlated with the number of dominant genes involved. Thus in the diploid the homozygous dominant is a slightly deeper pink than those plants heterozygous for this factor. Larger differences were found among the triploids where pinks of three genotypes are possible (*PPP*, *PPp* and *Ppp*). If the octoploid may be considered as having a replication of eight basically similar genoms, its gametes then carry four partially recessive factors for corolla color (*pppp*). In crosses with the tetraploid the two dominant genes (*PPpppp*) would thus be unable to function normally. Both the abnormal conditions of the hybrid plants and the possibility of an increased diluting or even inhibiting function of the "recessives" from the out-cross help to explain the results obtained.

Three families of the cross pistillate commercial variety × the tetraploid were grown. They likewise showed variation in leaf shape and vigor. Most of the plants proved to be completely sterile, although one plant of the cross Hunterdon × 223–6 set seed when unprotected out of doors. Four plants resulted which showed considerable variation in leaf shape, size, texture and coloration, in the size and type of serrations and in general vigor.

Cytology of the crosses

The cytological material obtained from these crosses, with the exception of that from the triploids, has been very limited. A single bud from the cross with *elatior* gave around 21 bodies at first metaphase in the pollen mother cells. It is quite possible that this represents seven bivalents from the seven pairs of homologous chromosomes of the tetraploid and likewise autosyndesis between two of the three genoms contributed by *F. elatior*.

In crosses between the octoploid species and the tetraploid, first metaphase counts varied from 21 to 28, with more counts approaching the former than the latter number. There is nothing to indicate whether autosyndesis occurs here or not. In crosses between the diploid and octoploid both types of association occur in the F_1, so that either assumption may be correct. The movement of the chromosomes to the poles is very irregular. Root tip counts made on two of the plants grown from seed, of the cross Hunterdon × 223–6 set out of doors, gave 42 and 40 somatic chromosomes respectively. Two other plants, from their morphological appearance, will certainly be found to vary widely from the above two plants in their somatic chromosome counts.

Changes in compatibility with chromosome doubling

The tetraploid Fragaria represents a case in which a doubling of the chromosome number has occurred without loss of fertility with the parental diploid species. This does not mean, however, that the crossing relationships of the tetraploid are identical with those of the diploid. There have, in fact, been changes in compatibility in two important points. Numerous attempts, previously reported, to obtain hybrids between *F. elatior* and various diploid species resulted in complete failure, although MANGELSDORF and EAST (1927) succeeded in getting four true hybrids that died in the seedling stage. In contrast to this, when the tetraploid was used as female, in crosses with *elatior*, the resulting plants were more vigorous than either parent. The 65 inviable seeds obtained in the reciprocal cross indicate a possibility of fertilization, although they may have been pseudogamic in origin. It is also possible that sterility may be due to conditions in the stigma being unfavorable for the growth of certain types of pollen. Thus BUCHHOLZ and BLAKESLEE (1929) find that the styles of triploid Datura are a very favorable media for the growth of pollen from the diploid, but very unfavorable for the growth of pollen from tetraploid plants.

These results do not fit into the scheme recently discussed by THOMPSON (1930) who shows that where there is a difference in the compatibility of reciprocal interspecific crosses involving different chromosome numbers, the one involving the species with the higher chromosome number as female is usually more successful. This is considered to be due to the fact that in this case the extra chromosomes are present in duplicate in the endosperm, whereas in the reciprocal they would be single. The above Fragaria crosses are thus not only an apparent, but a real exception to this general rule.

The other striking difference between diploid and tetraploid is seen in the complete failure of the cross tetraploid × octoploid. The diploid crosses readily when used as female with the octoploid. Likewise octoploid × tetraploid is easily accomplished. Partial fertility has been observed in this last cross as is true of certain octoploid × diploid crosses. The partial fertility in these cases may be due in part to pseudogamy but also results from the production of viable gametes with new chromosome numbers.

Unexpected chromosome numbers

Three cases of plants with unexpected chromosome numbers were found. A plant with 35 somatic chromosomes appeared in the selfed progeny of

one of the tetraploids. This plant was very similar to one of the types lacking a chromosome, to be described later. It was quite weak and died after setting one or two small fruits. The leaves were small and somewhat curled, and the terminal leaflet was either greatly reduced or entirely missing. Since the chromosome number falls within the polyploid series and since its appearance is that of an aneuploid type, the one or more missing chromosomes responsible for its form must have been replaced by non-homologous chromosomes that could not restore the normal balance.

Since twenty-seven and twenty-nine or thirty chromosome plants are not rare among the selfed progeny of the tetraploid, it seems likely that the 35-chromosome plant arose through the union of a 20- or 21-chromosome gamete, the result of some unusual distributional irregularity with a gamete carrying the diploid number or thereabouts. Five seeds from flowers of this 35-chromosome plant, open pollinated in the greenhouse, proved to be viable. One of the plants died at a very early stage; another of the same type as the parent died before blooming. Chromosome counts were not obtained for either of these plants. Of the remaining three, one has 27 somatic chromosomes and is unlike any of the types lacking a chromosome to be described later. The other two have 29 chromosomes each, and seem to belong to the extra chromosome types C and E, also described in the following section.

A plant from the cross *F. virginiana* No. 27, (pistillate) × 223–2 was also found to have 35 somatic chromosomes. It is only moderately vigorous and exhibits characteristics from both parents. It may be the result of the union of a haploid pollen grain from the tetraploid parent with the normal egg of *virginiana*. The plant is entirely sterile.

In a cross with the tetraploid using another pistillate form of *virginiana* as female, a plant having 38 somatic chromosomes was found. This plant, while quite vigorous, differs widely in appearance from its sibs. The leaflets are almost round and are very dark green. The serrations are smaller than and the leaves differ from its sibs in texture. It has not yet come into bloom. Its origin can probably be ascribed to the production of a 10 chromosome gamete by the tetraploid.

The second 35-chromosome plant described above is very similar to the group of unusual polyploid forms previously described (YARNELL 1931). While such forms have never been observed among the seedlings of pure species, there is evidently a tendency among species hybrids, shown especially in the group described earlier, to form plants with a chromosome number in the polyploid series and presumably consisting of complete chromosome sets.

THE ABERRANT CHROMOSOME TYPES

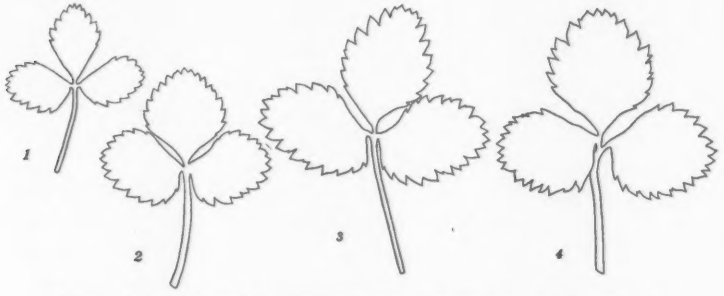
Origin

When the triploid families, grown for a study of the segregation of flower color in the tetraploid, were first transplanted from the seed pan, it was evident that certain of the seedlings varied greatly from the normal type. As the plants developed, these differences increased. A number of such abnormal types were in evidence, and chromosome counts from the root tips disclosed that all such plants possessed either a chromosome in addition to the 21 expected, or lacked a chromosome. Since the four genoms of the tetraploid may be supposed to be quite similar genetically, the three "homologous" chromosomes of each set of the triploid might be expected to be responsible for the same abnormal appearance of the plants whenever in excess and likewise whenever deficient. On this assumption seven $3n+1$ types are possible and also seven $3n-1$ types. Seven $3n+1$ types have been recognized and four $3n-1$ types thus far. The first group has been designated by the first seven letters of the alphabet, the other by the last four. Cytological evidence already presented makes it clear that the aberrant plants have resulted either from non-disjunction or loss of chromosomes into the cytoplasm during meiosis in the tetraploid.

Descriptions

The two species entering into the cross producing the tetraploid are quite similar in appearance. They are interfertile and give a completely fertile F_1 (MANGELSDORF and EAST 1927). The leaves of *F. vesca* are medium to large in size, varying with the cultural conditions. In this respect individual plants of *vesca* and its derivatives seem to vary more than do the other species of Fragaria. The leaves in potted plants attain a maximum size during the first year, those produced by the old crown in the greenhouse in subsequent seasons being materially reduced, especially during the winter months. An outline of a *vesca* leaf may be seen in text figure 2. All outlines are traced from a blueprint of the leaf and are reduced one-half. The serrations of *vesca* range in size from medium to small, and are roughly proportional to the size of the leaf. Most terminal leaflets have from 6 to 8 serrations on each edge; 5 and 9 are occasionally found. They are curved and slightly tipped. There is a definite pubescence on each surface of the leaf; it is somewhat heavier beneath. The petiole is heavily pubescent. The petiolules are very short. The flowers are medium to small; petals are large, either pink or white. The stamens are more conspicuous than the rather small pistil.

While *F. bracteata* is very similar to *F. vesca*, the two can be readily distinguished. The leaves are in general somewhat smaller than those of *vesca*, and are narrower in proportion to the length (see text figure 1). The serrations are similar to those of *vesca* in size, number and in shape, except that the tip is longer in *bracteata*. The flowers of both are also similar, but the petals of *bracteata* are a little longer than those of *vesca* and are always white. In *bracteata* the petiolule of the terminal leaflet is slightly elongated. This together with the narrower leaves usually gives more space between the leaflets.



FIGURES 1–4.—Balanced chromosomal types. 1. *Fragaria bracteata*. 2. *F. vesca*. 3. The triploid. 4. The tetraploid.

The first generation between the above two species is intermediate in most of the characters.

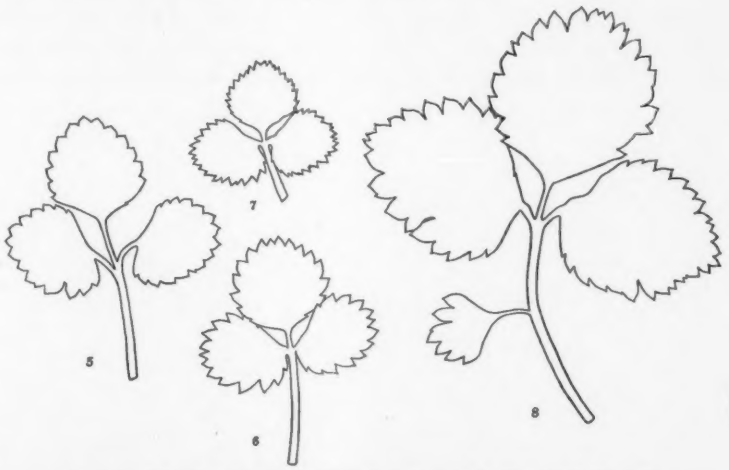
The tetraploid family 223 shows uniform and rather striking characteristics. As will be noted in text figure 4 the leaves exhibit an increase in size over those of the diploid; the serrations are coarser and the petiole thicker. Instead of being flat as in the diploid, the leaves are crinkly with a conspicuous venation. The flowers are also larger, although the fruit, evidently due to reduced fertility, is not as large as in the diploid. One of the seven plants of family 223 is much weaker than the others. Its leaves are smaller, although otherwise similar. The flowers are slightly smaller and practically female sterile.

While the triploid can be said to be intermediate between the diploid and tetraploid it more nearly approaches the latter. The outline in text figure 3 shows that the leaves are hardly as full as those of the tetraploid, but the serrations are nearly as coarse. The terminal petiolule is perhaps slightly longer than in *bracteata*. The flowers closely resemble those of the *vesca* parent, the color depending on the segregation of the factor for pink flower in the tetraploid.

3n+1 types

Type A is distinguished from the normal triploid by three striking characters—length of petiolule, shape of leaflets and shape of the serrations. All three petiolules are much longer than those of either triploid or tetraploid (text figure 5). The leaflets are short in proportion to width but do not appear round. The serrations are wide and shallow, well curved and tipped. The foliage is slightly darker than in the triploid. The flowers are somewhat smaller than those of the diploid, but the pistil is large compared with the size of the stamens. The type may be divided into two groups on a basis of leaf size and length of petiole.

Type B (text figure 6) is nearer the triploid than is A. It has two distinguishing features however. (1) In this case only the terminal petiolule is appreciably elongated. (2) The leaflets are nearly as broad as they are long, the terminal leaflet appearing round. Both serrations and flowers are similar in size and shape to those of the triploid.



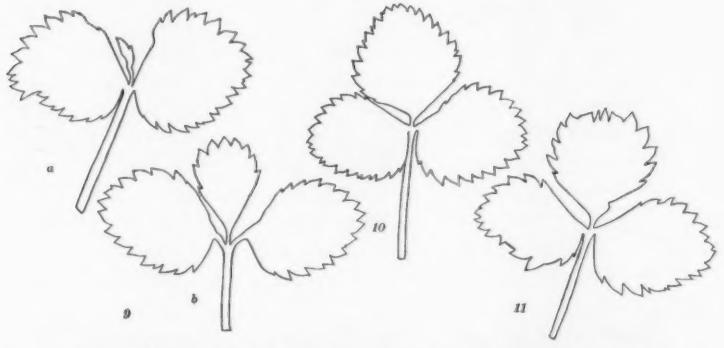
FIGURES 5–8.—3n+1 types. 5. Type A. 6. Type B. 7. Type C. 8. Type D.

Type C is hardly as vigorous as the normal triploid, the leaves being fewer in number. The terminal leaflet is often broader than long. The chief characteristic is the type of serrations which are very numerous, shallow and acute, being prominently tipped. They give a jagged appearance to the leaf (see text figure 7). The pubescence is most conspicuous at the edges of the leaves and on the petiole. The leaves are thick and rather stiff. The

petiole is of moderate length and is thick. The petiolules are short and heavy.

Type D is by far the largest and most vigorous of any, considerably exceeding the tetraploid in size. The leaves produced later in the season average hardly as large as the one illustrated in text figure 8. All, however, have serrations that are coarser and more numerous than those of the tetraploid. Many have supernumerary leaflets on the petiole. The petiolules, in common with the other features, are large, the terminal exceeding the laterals in length. The petiole is stout and long. The pubescence is about that of the diploid *vesca* in amount. The petals are as large or larger than those of *bracteata*. The pistil is large, but the stamens are slightly reduced. It is the only type to produce progeny when selfed, thus exceeding the triploid in fertility.

In type E a number of the terminal leaflets are either much reduced or entirely absent (text figures 9 a and b). Some of the leaves show no discernible reduction of the terminal leaflet. The lateral leaflets are always large. The serrations are coarse and tipped. The petiolules are very similar to those of the preceding type, but the terminal petiolule is not as long. The petiole is long and heavy. The flower is reduced in size, especially the petals and stamens.



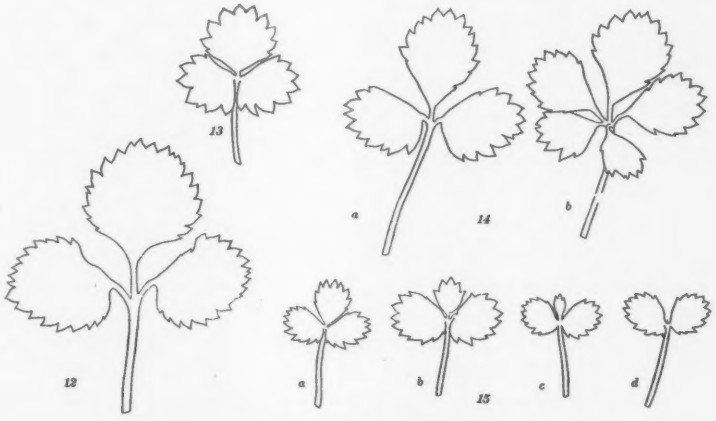
FIGURES 9–11.—3n+1 types. 9. a and b. Type E. 10. Type F. 11. Type G.

As may be seen by comparing text figures 10 and 3, type F differs little from the normal triploid. There is a slight increase in the number of serrations, and the leaflets are wider in proportion to length, but the size of the leaves remains about the same. The flowers are very similar to those of the triploid. In type F, however, the petals become somewhat elongated.

Type G (text figure 11), in common with E, may have the terminal leaflet reduced, although to a lesser extent. In addition, one of the lateral leaflets may be partially reduced instead of the terminal. Where the terminal leaflet is fully developed it is usually longer than wide. The leaves are rather stiff and glossy, dark green above and light underneath. The plants are much less vigorous and stalky than those of type E. The serrations are of medium size and tend to be acute. The petiole is only moderately heavy and is lightly pubescent. The flowers are about as in the diploid *vesca* and are completely sterile.

3n−1 types

The petiolules are long in type Z. The plants are somewhat similar in appearance to those of type A. The serrations, however, are very different, being deep and acute, and more numerous than in A. The base of the terminal leaflet as shown in text figure 12 is typical. The petiole is heavy and long. The veins are conspicuous and the pubescence is slight on the leaves but heavy on the petiole. This type has set seed unprotected out of doors.



FIGURES 12–15.—3n−1 types. 12. Type Z. 13. Type Y. 14 a and b. Type X. 15 a, b, c and d. Type W.

Type Y tends to dwarfness. The leaves are small and relatively few in number, but the serrations remain large and well formed. It grows very slowly and has been slow to bloom. The leaves produced the second season are larger than the one shown in text figure 13 and approach the normal form of type X.

Before plants of type X had reached maturity many or most of the leaves had what appeared to be a division of one or both lateral leaflets (text figures 14 a and b). This was a very constant characteristic. Later leaves, however, have only three leaflets which show a striking similarity to the *bracteata* type. The serrations are deep but not especially numerous. The flowers are small, the proportions about those of *vesca* flowers. Viable seed has been obtained with pollen from the diploid.

Type W is the least vigorous of the aberrant chromosome types. The leaves are small and the terminal leaflet is often reduced or entirely absent, as may be seen in text figures 15 a, b, c and d. The serrations are deep and almost acute. The flower is very small, although the petals are disproportionately large. It seldom blooms.

TABLE 2

Frequency and distribution of aberrant types.

TETRAPLOID SOURCE	3N+1 TYPES							3N−1 TYPES	TOTAL POPULATION
	A	B	C	D	E	F	G		
223–1	3	..	..	..	1	1	1	(0)	50
223–2	2	..	..	..	..	1	..	Z (1)	58
223–3	3	2	..	..	..	..	..	Z (1)	47
223–4	1	..	1	1	..	2	..	X (16)	48
223–5	4	..	..	2	1	..	2	Y (1)	98
223–6	..	..	..	..	..	..	..	W (1)	32
233–7	3	..	..	..	..	..	..	X (1)	67
Total	16	2	1	3	2	4	3		400

Frequency and distribution of types

It will be observed from table 2 that one tetrasomic and one disomic type are considerably in excess of the others. It might be supposed that the same chromosome was involved in each case, the result being due to the equal functioning of both gametes after non-disjunction. Type A is pretty well distributed among the progeny of the seven tetraploids while type X appears from only two, the great majority of the type X plants (16 out of 17) coming from crosses involving plant 223–4, which produced only one type A plant. The chromosomal complex of the gamete responsible for the production of type X seems to give it an advantage over the normal diploid pollen, since one-third of the progeny of the cross, diploid × 233–4, proved to be of this type, and it is unlikely that one-third of the male cells were lacking this particular chromosome. In an effort to find which chromosome was missing from type X and to learn something of its origin, somatic

chromosome counts were made on sixteen additional plants of the same cross. Two 22-chromosome plants, identified as types F and D were found, indicating that the type X plants were the result of chromosomal loss rather than of non-disjunction. Since only four 3n – 1 types were identified, it is possible that the chromosomal balance remaining when each of four non-homologous chromosomes of the set are missing gives the same or very similar morphological results. Anything, then, increasing chromosomal loss would increase the ratio of type X plants, especially if such pollen has the advantage postulated. This seems rather unlikely, however.

Other types appear in approximately equal numbers.

All of the extra chromosome types and all but one of those lacking a chromosome were carried through the pollen. Since most of the triploids resulted from crosses involving the tetraploid as male parent, the chance for aberrant chromosomal types occurring through the pollen was much greater than through the egg.

Discussion

While the effect of an unbalanced chromosomal complement is definite and specific, it should not be overlooked that the general appearance of the plant is likewise dependent upon environmental conditions, such as water, temperature and sunlight. Age is also a factor in influencing appearance. The descriptions given are for plants approaching the maximum growth where conditions for light and temperature have been good. Some of the changes ordinarily found under poor conditions have been noted above, size of leaves being the most conspicuous. Under poor conditions types B, D and F approach the triploid in appearance under similar conditions; the others can be quite readily identified even under these circumstances. Different individuals of the same type show small variations, as do the triploids, due probably to different combinations of *bracteata* and *vesca* chromosomes which are similar but not identical.

The effect of the extra chromosomes in combination with three complete sets may be summarized as follows:

Chromosome A: an increase in length of all three petiolules, the leaflets being of medium size and nearly as broad as long, and the serrations wide and shallow.

B: an increase in the length of the terminal petiolule only; the terminal leaflet is nearly round, and the serrations are somewhat enlarged over those of the triploid.

C: the leaves are quite stiff and the number of serrations is greatly increased.

D: increased growth and fertility results.

E: the size is increased and the balance necessary for the normal development of the terminal leaflet is upset.

F: the normal balance is changed only to a very slight extent.

G: the amount of pubescence is reduced and the normal development of all three leaflets is interfered with.

With these points in mind, the particular chromosome lacking in the 3n−1 types may be considered.

Type Z shows the influence of chromosomes A, B and C. The full development of all three leaflets suggests the absence of E or G.

Type Y seems to lack the growth stimulation of chromosome D.

Type X shows some of the characteristics associated with chromosomes A, B, D and G. Chromosome C may be absent in this case.

Type W shows the influence of chromosomes B, C, E and G. The absence of chromosome A is suggested here.

The above assignments of the particular chromosome absent are, of course, entirely tentative. An effort was made, by a careful study of the somatic chromosomes of the root tips, to identify cytologically the chromosome associated with each of the above types. As explained in an earlier paper (YARNELL 1929a), the only constant difference between the somatic chromosomes is one of relative size. Since the average range for the haploid set is only from 0.9–1.8 microns, the determination of which of the seven chromosomes was tetrasomic in a 22-chromosome plant could not be made with any degree of confidence.

The loss of a chromosome from the 3n balance seems to cause more of an upset than the addition of an extra one. All seven of the extra chromosome types have been found among the tetraploid progeny, but only four types lacking a chromosome. Of the latter group two are somewhat below the normal triploid in vigor and a third (W) is very weak. None approaches the normal as does type F. The fact that fewer 3n−1 types than expected appear must be significant, since non-disjunction should produce equal numbers of each type of gamete and, in addition, chromosomes lost in the cytoplasm should increase the amount of 13-chromosome pollen. It is not known whether the lethal effect is gametic or zygotic in these cases.

Certain aberrant types with higher chromosome numbers have appeared. Two plants varying only slightly from type A were found to have 23 somatic chromosomes. The identity of the second chromosome involved has not been ascertained. The characteristics of type A are not more marked; neither are the distinguishing features of any other type apparent. It may be that the second chromosome is F or perhaps one which has its

own characters balanced by the extra chromosome A. FROST (1927) finds two cases of combined trisomic types in Matthiola in which the characteristics of both are evident. A plant with three chromosomes extra he supposes to have two chromosomes of one type and one of another, since it was derived from a 2n+1+1 plant showing their combined features. It is interesting that abnormal chromosome numbers in *Zea mays* range from 21 to as high as 28 (RANDOLPH 1928) without striking changes in appearance, although MCCLINTOCK (1929) finds a decrease in vigor and fertility with an increase in chromosome number.

In a backcross to the diploid in which the tetraploid Fragaria was used as female, a "maternal" appeared that has 29 somatic chromosomes. It corresponded very closely to type B. Two 29-chromosome plants were found in the progeny of one of the tetraploids selfed. One belonged to type B, the other to type E.

NEWTON and PELLEW (1929) report the appearance of three abnormal types derived from the tetraploid *Primula kewensis;* two had 35 and one had 37 chromosomes. Both 35-chromosome plants produced a new form with 34 chromosomes. The first three types, when selfed, did not produce normal tetraploids. This is attributed to intraspecific chromosome pairing normally, with interspecific pairing with interchange between chromosomes giving rise to new types.

A self-fertile "maternal" of unknown chromosome number of the cross 223−5×*F. americana alba* gave plants when selfed with the following somatic chromosome numbers:

Chromosome number:	15	26	27	28	29	30
Number of plants:	1	2	2	7	5	2

Those with 28, the tetraploid number, correspond very closely to the normal type. The 15-chromosome plant is of type C. One 26-chromosome plant is very close to type W, but more vigorous; the other plant has not yet been identified. One of the 27-chromosome plants is small and can probably be referred to type Y; the other is new. Of the 29-chromosome plants one is type B, two type E and two probably type G. Both 30-chromosome plants seem to be a combination of E and F. The above types are somewhat more vigorous than the corresponding ones of the triploid but are otherwise very similar.

A backcross to the diploid of a type B 29-chromosome plant, which is a sib to the parent of the above family, includes plants of the following types: two 20-chromosome plants classified as type X, ten normal triploids, four 22-chromosome plants (one A, one B, one F and one G), and two 23-

LEGEND FOR PLATE 1

All drawings were made with the aid of the camera lucida, using a 1.5 mm Spencer apochromatic objective, with ×18 ocular and a tube length of 160 mm. They have been reduced one-fourth in publication. Figures 1–11 are of pollen-mother cells.

FIGURES 1 and 2.—Tetraploid×*F. collina;* prophase showing two nuclei.

FIGURE 3.—*F. americana alba*×tetraploid; diakinesis.

FIGURE 4.—*F. americana alba*×tetraploid; 3n+1 type B, 11 disomes.

FIGURE 5.—*F. americana alba*×tetraploid; 10 bivalents+1 univalent.

FIGURE 6.—*F. americana alba*×tetraploid; 3 tetrasomes, 3 disomes and 1 trisome.

FIGURE 7.—Tetraploid×*F. sp* FPI 64856; second metaphase showing a 10+11 distribution.

FIGURE 8.—*F. americana alba*×tetraploid; early first metaphase, 3 tetrasomes, 3 disomes and 1 trisome.

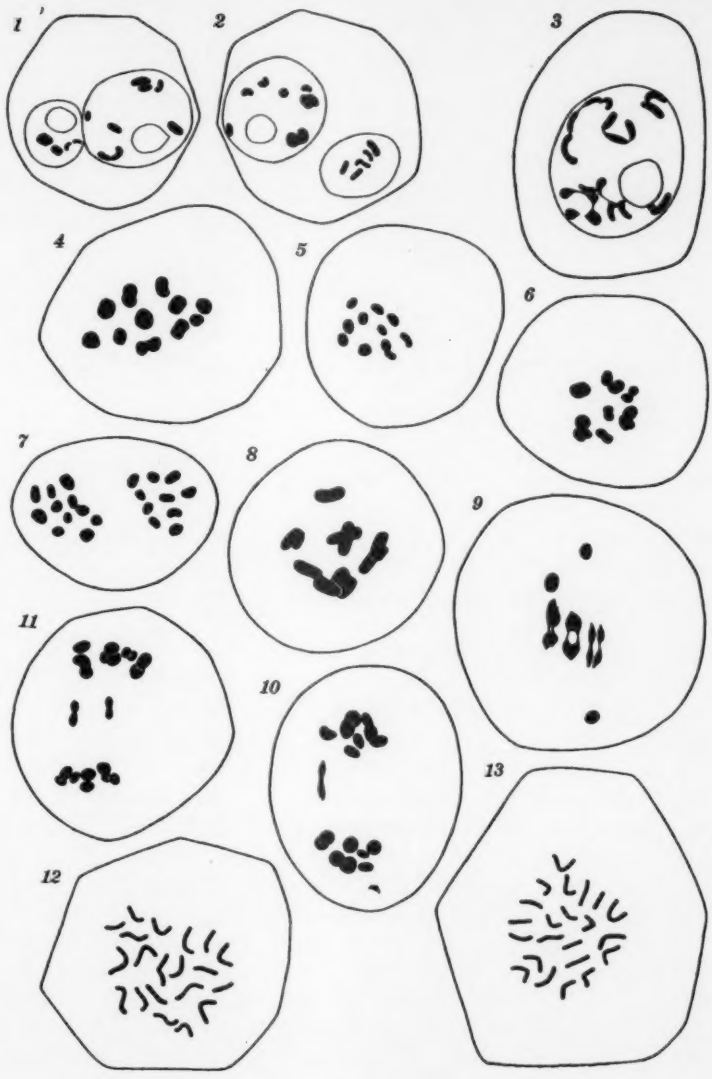
FIGURE 9.—*F. americana alba*×tetraploid; spindle view of first metaphase showing two tetrasomes.

FIGURE 10.—Late anaphase of the tetraploid showing chromosome lagging.

FIGURE 11.—*F. americana alba*×tetraploid; lagging at first metaphase.

FIGURE 12.—*F. americana alba*×tetraploid; 3n−1 type X, somatic division from root tip showing 20 chromosomes.

FIGURE 13.—Same family as figure 12; 3n+1 type C, 22 somatic chromosomes.


1
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12

chromosome plants both having chromosome B extra, one in combination with chromosome E and the other with either E or G.

MEIOSIS IN THE TRIPLOID

The only evidence of irregular behavior in prophase stages preceding diakinesis is found in the F_1 of the cross tetraploid×*F. collina*, in which occasionally two nuclei can be seen in a single cell. Figures 1 and 2 of plate 1 show two pollen mother cells, evidently of common origin, each containing two dividing nuclei at diakinesis, three of the nuclei each showing a nucleolus. One is about twice the size of the other. It is possible that the chromosome set from *collina* is organized in the small nucleus and that two sets from the tetraploid form the larger one. While numerous similar cases have been observed at earlier stages, there is no evidence of such a separation either previous to or later than the meiotic prophases, and no evidence from mitosis in the root tips.

It is often difficult to make out the precise relationships of the chromosomes at diakinesis, since it is not always certain that proximity has any real significance. Figure 3 illustrates what is believed to be about the maximum amount of association at this stage. It includes two pairs, one trivalent group and three groups of four. The nucleolus, which is stained very deeply, is assumed to hide a third pair. The group in the upper left consists of three chromosomes of nearly equal size together with one much larger.

The most interesting feature of meiosis in the triploid is the consistent complete pairing of the chromosomes at the heterotypic metaphase. Often there is also a further association among the eleven pairs, which results in a count of seven at I M. This was first interpreted as seven trisomes, a type of association found in so many triploids. (A list of such forms is given by McCLINTOCK 1929.) Further examination, however, revealed that three were tetrasomes, three disomes and only one a trisome. Such an arrangement can be easily recognized in figure 6 in which the association is unusually loose. The trisome is below to the left; the three disomes are below, center and upper right, and the tetrasomes lower right, upper middle, and left. The one in the upper middle has nearly separated into two disomes. A slight pressure on the coverglass serves to separate the two parts of the tetrasome in many cases. Another first metaphase plate is shown in figure 8. Here the trisome is at the left with two disomes below and one at the top. In figure 9 may be seen a spindle view of early anaphase. Early separation of a few chromosomes is typical. On the plate at the left is a tetrasome involving three chromosomes of equal size and one somewhat larger and of

a different shape. A number of chromosomes have been omitted from the group. Figure 11 shows lagging chromosomes, a phenomenon more frequent in the triploid than in the tetraploid. In figure 7, a 10–11 segregation has occurred during the first division and the chromosomes are lined up quite regularly on the homotypic plates. Other cases exhibit extrusion of chromosomes into the cytoplasm, lagging and reduced numbers.

The tetrads appear normal for the most part; it is only very rarely that more than four microspores are formed. This is in considerable contrast to what is found in the pentaploid hybrids; the difference may be due to the amount of chromatin available for a micronucleus.

Complete pairing of the chromosomes has been observed in all of the material examined. Fresh aceto-carmine mounts gave the same counts as buds sectioned in paraffin. The latter were fixed for the most part in Allen's modification of Bouin's fixative, which gave excellent results, and also in Navashin's chrom-acetoformaldehyde solution.

Unfortunately it was impossible to determine the stage of meiosis at which pairing between non-homologous chromosomes begins, except that it was sometimes seen at diakinesis. This may be taken as an indication of association at earlier stages with the concomitant possibility of crossing over between such chromosomes, or it may be more superficial. NEWTON and DARLINGTON (1929) in describing meiosis in triploid tulips say, "At the end of diplotene, and in the ensuing stages up to metaphase, association between chromosomes is thus maintained only by exchanges between their constituent halves, the chromatids" (page 6). Such a cause of association, if applicable, would seem to favor the first possibility.

Doctor KARL SAX suggests in a private communication that this pairing of non-homologous chromosomes may be of the nature of that found in XY pairing in Drosophila. Here pairing of the X and Y chromosomes is loose and no crossing over occurs either in the male or female.

The constant formation of three instead of four tetrasomes has been given the following explanation. If the three genoms of the triploid be represented thus: A B C D E F G, a b c d e f g and A_1 B_1 C_1 D_1 E_1 F_1 G_1 we might expect any two sets to pair in the usual manner: Aa, Bb, Cc, Dd, Ee, Ff, and Gg (or AA_1, bB_1 etc.). Since there is complete pairing, the third set would give association of non-homologs: A_1B_1, C_1D_1, E_1F_1 and G_1. Where secondary association occurs one might expect disomes of the second group to associate with a homolog of the first, which gives, for example, AaA_1B_1, CcC_1D_1, EeE_1F_1 as the three tetrasomes. The trisome would then consist of GgG_1, leaving the three disomes Bb, Dd and Ff with

nothing in common to effect a secondary association. (If the association were A_1B_1Bb, then Aa would remain a disome.)

Reference has been made in an earlier paper (YARNELL 1929b) to a similar method of pairing generally effective in two Oenothera hybrids, reported by GATES (1909 and 1923). The triploids arose from crossing *O. lata* and *O. rubricalyx* with *O. gigas* as pollen parent. He finds considerable irregularity, however, so that, "pollen grains will be formed having every chromosome number between 11 or 12 and 7" (GATES 1923). GEERTS (1911) studying an Oenothera triploid from the cross *Lamarckiana-gigas* found only seven pairs plus seven univalent chromosomes at the heterotypic metaphase. Since the cause or causes of pairing of non-homologous chromosomes are as yet unknown, there is no reason to expect triploids of unlike origin in the same genus to behave identically in this respect.

LONGLEY (1924) points out the similarity of chromosome behavior in the triploid Oenothera and in the triploid Rubus species studied by him. Both have 21 somatic chromosomes and both have shown 10 and 11 bodies at first metaphase.

Another case of similar association during the meiotic mitosis has been found in a triploid Citrus (LONGLEY 1926). In this instance only a single individual was investigated. It resulted from the triple cross (*Fortunella margarita*×*Citrus aurantifolia*) (9) ×*F. hindsii* (18), giving a plant with 27 somatic chromosomes. He found mostly 13 bivalents plus one univalent at I M with 13 chromosomes at II M. There was little tetrad irregularity. NISHIYAMA (1929) reports conditions in a triploid resulting from the cross *Avena barbata* (14) × *A. strigosa* (7). He finds pairing between two non-homologous chromosomes in 25 percent of the 100 first metaphase plates examined. Reference is made by TISCHLER (1929) to cytological observations of CHIARUGI (1926) on the triploid *Artemisia nitida*. Both 9 bivalents plus 9 univalents, and 9+4 bivalents plus 1 univalent were found at first metaphase. Four of the bivalents in the latter case would represent pairing between non-homologous chromosomes.

There are numerous cases in which species with different chromosome numbers are crossed (often in the ratio of 2:1) in which complete pairing among the chromosomes at meiosis has been observed, but the larger number of chromosomes involved invites the assumption of autosyndesis between two related groups rather than association between chromosomes ordinarily considered non-homologous. The instances cited above include only triploids with chromosome complements of small prime numbers. It is realized that this does not preclude the possibility of homology among

the chromosomes of a set, but the assumption seems reasonable, especially where the haploid number is the basic one for the genus.

Such observations are significant for theories of chromosome pairing of general application. A considerable amount of cogent evidence has accumulated showing not only consistency of pairing between morphologically similar chromosomes, but also showing that contact is between *like* ends of chromosomes that associate. Thus in the Datura 2n+1 primaries the trivalent usually forms a chain, while in the 2n+1 secondaries a closed ring of three chromosomes is found (BELLING 1927). The new configuration is assumed to be possible because of "reverse segmental interchange" between two homologous chromosomes, giving a new type with similar ends.

Demonstration of pairing between non-homologous chromosomes has no effect upon the validity of such observations and conclusions, but may somewhat limit their universal application.

DARLINGTON in a paper on Prunus concludes that "differences in genetic properties are associated with, if not responsible for, variations in affinity of homologous chromosomes" (1928, p. 239). He includes single factor differences as well as more general genetic relationships. It is quite possible that factors other than the degree of genetic relationship may likewise decrease the amount of pairing among homologous chromosomes. DARLINGTON further cites the work of HUSKINS (1928) on fatuoid oats in support of the supposition that the non-pairing of certain chromosomes may be due to a single gene. SHARP (1926, p. 280) in referring to extra chromosome forms in *Zea mays* cautions against "attributing lack of synapsis to absence of homology."

BEADLE (1930) has found a case of complete lack of pairing in *Zea mays* due to a single recessive gene. There seems to be no possibility here of reduced homology. GOWEN'S case of non-crossing over in Drosophila (1928) is probably of a similar nature.

In studying the cytology of progeny from X-rayed sex cells, GOODSPEED (1929) describes five plants as showing a variable amount of conjugation, a part of the cells giving 24, the normal number of bivalents at first metaphase. It would seem that induced conditions other than non-homology might here be responsible for the wide variability in association.

KOSTOFF (1929) attributes irregularities in the meiotic divisions of *Nicotiana Tabacum macrophylla* and *N. Langsdorffii* to the influence of the stock, when scions of these Nicotianas were grafted on *Datura Wrightii* and *Solanum nigrum* respectively. A reduced amount of pairing in *Lycium*

halimifolium due to the attack of a gall mite has been observed by KOSTOFF and KENDALL (1929).

The fact that conditions other than homology seem to induce association of non-homologous chromosomes may also be assumed to apply to chromosomes of various degrees of homology as obtaining in species hybrids. Such considerations reduce confidence in an assumption of a complete positive correlation between chromosome affinity and genetic relationship.

The limited experience thus far obtained indicates three possible influences on the pairing of chromosomes apart from their genetic identity: (1) single gene mutations, (2) abnormal conditions due to hybridization and (3) the primary or secondary effect of a number of influences including X-rays, grafting, temperature, etc. The second appears to be of greatest importance where inferences of genetic relationship are drawn from chromosome behavior.

THE TRIPLOID DERIVATIVES

Fertility in the triploid

So far as is known, the triploids have never spontaneously set fruit in the greenhouse, though the pure species and the commercial types with perfect flowers set fruit freely without artificial pollination. Small fruits have been obtained frequently on crossing the triploid reciprocally with the diploid. Open pollinated flowers will also set out of doors, although only a small number of achenes develop. Tetrasomic D and possibly E have set fruit in the greenhouse without artificial pollination, but not under conditions excluding pollen from other sources. When the triploid was pollinated with pollen from the diploid, 30 of the 80 pollinations, or 37.5 percent, set fruit. The 378 seeds obtained gave about 18 percent germination, a total of 71 plants being raised. From the reciprocal cross 4 out of 23 pollinations set—approximately 17 percent. The 99 seeds obtained gave about the same percentage of germination as before. Thus in spite of the fact that many more male gametes than female are involved in the crossings, with the accompanying selection of functional pollen, the triploid shows greater fertility on the female side.

The progeny

The various derivatives of the triploid and of the related abnormal types show a large amount of variability, due in part to differences in chromosome number. Plants strictly comparable in all respects are rare. A total of 36 plants with the diploid number of chromosomes appeared among these populations. Of this number 31 are classified as having the appearance

of the normal diploid. Fifteen of these plants have bloomed, seven were found to be completely fertile, three were sterile and five showed evidence of reduced fertility. Sixteen of the plants resembling the normal diploid have been slow in coming into bloom. This is also a characteristic of the abnormal types. Of the five remaining plants with the diploid number of chromosomes, three are very much alike in leaf shape and type of serrations, both of which are atypical. Two of these show considerably reduced vigor. One of the other plants is small, and the fifth, while normal in vigor, has leaves that are thick and heavy.

TABLE 3

Frequency of types in triploid derivatives.

CROSS	A	B	C	D	E	F	G	"NORMAL" DIPLOID	X	Ia	Ib	Ic	Id	MISCELLANEOUS
2n×3n	..	..	..	..	..	1	..	8	1	..	..	..	...	1
3n×2n	..	..	2	..	1	4	..	7	1	1	..	4	2	4
2n×3n+1	..	..	..	..	..	..	..	3	..	..	..	..	..	..
3n+1×2n	1	..	1	..	2	1	1	4	2	..	3	1	..	8
3n+1 selfed	..	..	..	..	..	1	..	2	..	..	..	..	..	1
3n−1×2n	..	..	2	..	..	2	..	7	..	6	3	2	4	1
Total	1	0	5	0	3	9	1	31	4	7	6	7	6	15

The following easily recognizable types have been found among the 15-chromosome plants: A, one plant; C, four plants; E, three plants; F, eight plants, and G, one plant. One rather weak plant was classified as a modified type F. In addition to the above, twenty-four 15-chromosome plants could not be assigned to any previously known type. These plants will now be classified and given group numbers to distinguish them from the 3n+1 types already described. Twenty-one of these plants have certain characteristics in common, namely, leaflets that are narrow in comparison to length, and serrations that are of medium size but rather deep. There also is a tendency for the terminal leaflet to be reduced. This group, which will be referred to as number 1, can be separated into four subgroups that tend to merge. The first three have light green foliage, the leaves of the fourth being darker in color.

The other three 15-chromosome plants have each been placed in separate groups as follows: The plant forming group number 2 has leaves of normal shape with many acute serrations. It is very different in appearance from type C. The many veins are conspicuous. The plant is completely sterile. Group number 3 (one plant) is characterized by extremely stiff leaves. The leaflets are narrow and the terminal leaflet is often reduced. Group 4, also a single plant, resembles type G in having only a

slight amount of pubescence on the upper surface of the leaf. The leaflets are fully developed and are narrower than those of G. The last three groups could be considered as extremes of group 1.

Six 16-chromosome plants were found. One is of type F, four were referred to group number 1 and one remains unclassified. One 17-chromosome plant is of type C; another belongs to group number 1. Two 19-chromosome plants form group number 5. They are very similar to type W but are much larger. The leaflets are very misshapen, but the plants are fairly vigorous. The four 20-chromosome plants all belong to type X. Three normal triploids appeared. A single 22-chromosome plant was found which proved to be of type C.

A total of 80 plants have been grown from seed set on triploids out of doors without any protection from cross-pollination. Most of the types already described are found in these families and, in addition, a number that appear to be new. Certain of the extra chromosome types such as A and C appear in extreme form. Minor differences of many kinds are in evidence, and variability is the rule throughout these progenies. The chromosome number of most of these plants has not been determined.

Five of the extra chromosome types have reappeared in the progeny of the triploid. These 2n+1 plants, as might be expected, are not identical with the corresponding 3n+1 types. The 2n+A plant has smaller leaves than the corresponding tetrasomic, but the distinguishing characters, leaf shape, type of serrations, and length of petiolules remain the same. The leaves of the trisomic are slightly thinner but are of about the same texture. No well defined B or D types were identified among these plants. The trisomic type C has longer, less stocky petioles than its tetrasomic relative. The serrations are the most outstanding characteristic of the type and make recognition easy. The trisomic plants show differences in vigor and leaf texture, the less vigorous plants having stiffer foliage. The trisomic E retains the leaf shape common to the type. The leaves are thinner and the petiole is not so heavy. The nine plants placed in type F do not differ appreciably from the normal diploid, and their classification is due entirely to their somatic chromosome number.

It may be said that the characteristics associated with the presence of a chromosome in addition to the complete sets are not, in general, more pronounced in the trisomic than in the tetrasomic (3n+1) condition. The morphological difference between 2n+1 and 3n+1 is not dilution or intensification of the characters due to the extra chromosome so much as to differences attributable to an extra set of chromosomes in the one. Such differences, then, can be ascribed to the extra set rather than to a ratio be-

tween the extra chromosome and the number of sets. The characteristics due to the extra chromosome remain about the same: the major change is in leaf size, thickness of petiole, and thickness and texture of the leaves. This is in contrast to BLAKESLEE'S findings in regard to the Globe mutant of Datura (1922). He reports a considerable decrease in intensity of Globe characters with an increase in the number of full sets of chromosomes and finds that a 4n+2 plant is comparable to a 2n+1 individual in this respect.

The distribution of the extra chromosome types in the triploid progeny does not follow that of the tetraploid. In the latter case type A is far in excess, while in the former only one such plant appears. The 2n+1 type C is found oftener than expected in progeny from the triploid. This is also true of type F, the one nearest to the normal. No plant was identified as having 2n−1 chromosomes.

TABLE 4

Chromosome numbers of triploid derivatives.

SOMATIC CHROMOSOME NUMBERS SOMATIC NUMBER MINUS 7	14 (7)	15 (8)	16 (9)	17 (10)	18 (11)	19 (12)	20 (13)	21 (14)	22 (15)
2n×3n	9	1	..	..	..	..	1	..	..
3n×2n	9	11	2	1	..	..	1	..	1
2n×3n+1	3	..	..	..	..	..	..	..	..
3n+1×2n	5	12	..	..	..	2	2	3	..
3n+1 selfed	3	1	..	..	..	..	..	..	..
3n−1×2n	7	16	4	1	..	..	..	..	..
Total	36	41	6	2	0	2	4	3	1

Distribution of chromosomes

It will be observed from table 4 that different proportions of 14- and 15-chromosome plants are obtained from different crosses. Both triploid and aberrant types produce a larger number of the latter when used as female. The diploid used in the 2n×3n crosses was a pistillate form of *F. bracteata*, so the diploids in the progeny can hardly be due to accidental selfing. The chromosome numbers of the gametes received from the "triploid" parent (3n, 3n+1, 3n−1) are listed in the second line of the table. The chromosome numbers received by the successful gametes are obviously the result neither of a consistent 10+11 segregation nor of a chance distribution. It would appear that no 11-chromosome gamete had been successful and only two 10-chromosome gametes. Since reduction division has not been investigated in the egg mother cells of the triploid, it is impossible to say what has occurred there. A study of meiosis in the pollen mother cells re-

vealed considerable irregularity in chromosome distribution. Unsuccessful chromosome combinations may have been eliminated during gametic development, after pollination, after fertilization and upon attempted germination of the seed. FEDERLEY (1929) finds an unsuccessful chromosome combination in a species cross involving the sex chromosome. In the cross *Metopsilus porcellus*×*Chaerocampa elpenor* a normal sex ratio is obtained, but in the reciprocal all of the females die in the pupa stage, individuals receiving a *porcellus* X-chromosome and an *elpenor* Y-chromosome developing no farther.

Those plants with somatic chromosome numbers of 19 or higher, with the exception of a single 20-chromosome plant, may have arisen through some form of pseudogamy.

An excess of plants with complete or nearly complete sets of chromosomes is frequently found in progeny of plants having an odd number of genoms. NAVASHIN (1929) finds large numbers of 2n, 3n and even 4n plants from the triploid *Crepis capillaris* selfed, whereas the aneuploid numbers are relatively scarce. In addition, the trisomic type C did not appear at all. In the triploid derivatives of the *Raphanus sativus*×*Brassica oleracea* cross KARPECHENKO (1928) obtained upon selfing 75 percent 2n and 2n+1 plants. Triploid×the diploid *R. sativus* gave only diploids. Triploid×diploid tomatoes produced thirty-five 2n+1, fourteen 2n+2 and two 2n+3 plants (LESLEY 1928). Nine of the twelve primary trisomics possible were found in about equal numbers.

In contrast to the above, DARLINGTON finds that in triploid hyacinths every chromosome number between 16 and 30 seems to be equally successful (NEWTON and DARLINGTON 1929). The *Zea mays* triploids investigated by MCCLINTOCK (1929) when used as female with the diploid produced no diploids but only extra chromosome plants as follows: one 2n+1, three 2n+2, one 2n+3, three 2n+4, one 2n+5 and one 2n+7 plant. The reciprocal gave 27 diploids, six 2n+1, three 2n+2 and one triploid.

In a study of partial sterility in the *Nicotiana paniculata-rustica* cross LAMMERTS (1929) concludes that, "Sterility of the F_1 is evidently not primarily due to inviability of gametes resulting from random distribution of the univalents, inasmuch as viable gametes apparently represent a random sample, as respects the chromosome number, of the entire gametic series" (LAMMERTS 1929, p. 303).

In the F_2 and F_3 of pentaploid wheat hybrids SAX (1923) finds a similar situation. In addition, "The plants with 14 chromosomes were similar in morphological characters and rust resistance to the *durum* parent, while segregates with 21 chromosomes had the morphological characters and sus-

ceptibility to rust of the *vulgare* parent." THOMPSON and CAMERON (1928) report similar cytological results with another pentaploid wheat hybrid.

Causes of variation

Certain results observed in the progeny of the "triploids" require further explanation. These include the sterility found in a portion of the diploids, the unexpectedly large number of type F plants, the appearance of the twenty-one 15-chromosome plants in group one and the increased amount of variability in all groups.

Three things may be suggested as being responsible for the results in Fragaria: (1) new combinations of *vesca* and *bracteata* chromosomes, (2) the appearance of diploids lacking one chromosome and having one or two different chromosomes in excess and (3) crossing over between non-homologous chromosomes in the triploid.

Substitution of bracteata and vesca homologs

Since pairing among the four homologs of the tetraploid was found to be entirely fortuitous, it is reasonable to suppose that the same thing is true of the triploid. Gametes from the triploid would thus carry a great variety of combinations of *vesca* and *bracteata* chromosomes which would find expression in the progeny. The question then arises whether or not the differences between the homologous chromosomes of these two species are large enough to account for the new types found. Judging from their morphological similarity and complete interfertility, from the fact that only seven $3n+1$ types appeared, and from the additional fact that the triploids showed no such large differences, it seems that this point alone is hardly sufficient to explain the observations.

Substitution of non-homologous chromosomes

The second possibility seems to hold greater opportunity of definite change in the appearance of a plant. A $2n-1+1$ plant might be expected to show the characteristics of some observed type. It would also necessitate the assumption of a viable $n-1+1$ gamete which seems rather doubtful, although BLAKESLEE (1928) lists the appearance of a single $2n-1$ form in Datura. The morphological appearance of any particular $2n-1+2$ or $2n-1+1+1$[2] plant could not be predicted, since this involves a new balance. Such an assumption might account for the 15-chromosome group number one. If the homologous chromosomes of two genoms pair in the

[2] This refers to a loss of one and a gain of two non-homologous chromosomes.

triploid, each gamete should normally receive at least one complete set of chromosomes. Group 1 would then have to be accounted for on the basis of non-disjunction and chromosome elimination.

Segmental interchange between non-homologous chromosomes

The third possibility, that of crossing over between non-homologous chromosomes in the triploid, should be sufficient to explain the variaonsti observed in the progeny. There seems to be a cytological basis in the triploid for supposing an opportunity for such crossing over, although just the pollen mother cells have been examined. The implications are such, however, that further evidence would be necessary before such a theory could be accepted. It seems to explain the following facts: (1) the appearance among derivatives of the triploid of completely sterile plants, with the diploid chromosome number but unlike the diploid morphologically, (2) a reduced fertility in diploids entirely normal in appearance, (3) the production of twenty-one 15-chromosome plants of similar type, but varying among themselves, that could not be assigned to any of the known extra chromosome types and (4) the presence of marked variations among extra chromosome plants of the same type. No final decision as to the proper explanation can as yet be made.

SUMMARY

1. A study of the segregation of pink flower color in seven F_2 plants of the tetraploid shows that the chromosomes pair at random. Of these seven plants one was found to be triplex for the factor for pink flower color, two are duplex and four simplex for this factor.

2. Both chromosome lagging and chromosome elimination were observed during reduction division in the tetraploid.

3. The tetraploid differs from the parental diploid species in out-crosses with other species in two respects. The tetraploid gives vigorous but sterile plants when used as female with *F. elatior*, a cross that is impossible with the diploid. It has been impossible to produce the cross tetraploid×octoploid, while diploid×octoploid is rather easily obtained.

4. Seven distinct $3n+1$ types appeared when the tetraploid was crossed with the diploid. They were due to non-disjunction during reduction division in the tetraploid.

5. Four $3n-1$ types appeared among the progeny of the same cross. The production of these plants can be accounted for both by non-disjunction and by chromosome elimination in the tetraploid. They were shown to be independently derived.

6. The 3n+1 type A and the 3n−1 type X were found more frequently than the other types in these crosses.

7. Complete pairing was observed consistently in the pollen mother cells of the triploid. More than 10 or 11 bodies are never found on the plate at first metaphase. While a 10+11 division often results from this type of pairing, the irregularities, including chromosome lagging and chromosome elimination, are rather numerous.

8. A secondary association of disomes was also found to occur in the triploid.

9. Plants from triploid-diploid crosses varied both in chromosome number and morphological appearance. Many of the 15-chromosome plants could be identified as of the same type as the 3n+1 plants previously found. Others were new.

10. The 2n+1 and 3n+1 types differ chiefly in the same way that 2n and 3n plants differ. The distinctive characters due to the presence of an extra chromosome remain about the same.

11. The successful gametes of the triploid do not represent a random distribution of chromosomes at meiosis. A larger proportion of diploids appeared in the diploid-triploid cross than in the reciprocal.

12. Three suggestions are made to account for the sterility of diploids and the appearance of the new forms found among the triploid derivatives: (1) substitution of homologous *bracteata* for *vesca* chromosomes and *vice versa*, (2) substitution of non-homologous chromosomes, due to irregularities in the reduction division of the triploid, and (3) interchange between non-homologous chromosomes during pairing of such chromosomes in the triploid.

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A NEW TYPE OF TRANSLOCATION IN *DROSOPHILA MELANOGASTER*[1]

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INTRODUCTION

The first known translocation in Drosophila was the "Pale" translocation found by BRIDGES (1919, 1923) in which a piece from the end of the right arm of the second chromosome, including the genes from plexus to speck, had become attached to the side of the third chromosome near the locus of ebony. This, and a few other translocations found by BRIDGES, occurred spontaneously, that is, in a fly which had not been subjected to irradiation. Following the discovery by MULLER (1928a, 1928b) and WEINSTEIN (1928) that the frequency of translocations in Drosophila could be markedly increased through the agency of X-rays, a number of papers (DOBZHANSKY 1929a, 1929b, 1930a, MULLER and PAINTER 1929, PAINTER and MULLER 1929, MULLER and ALTENBURG 1930) have appeared presenting the frequency and the cytological and genetical characteristics of translocations.

In the published cases of translocations induced by X-ray treatment the broken-off piece of chromosome involved in the translocation was an end or terminal portion. However, DOBZHANSKY (1930b) found two translocations in which a piece had been taken from the middle region of the second chromosome and had become permanently attached to the Y chromosome. He designated these as translocations G and H respectively. Doctor DOBZHANSKY was so kind as to turn over his translocation G to the present author for analysis and this paper reports the results obtained. I wish

[1] This paper reports research conducted at the CALIFORNIA INSTITUTE OF TECHNOLOGY.

here to express my sincere appreciation to Professors DOBZHANSKY and STURTEVANT for advice and suggestions so freely given during the course of this investigation.

ORIGIN OF TRANSLOCATION

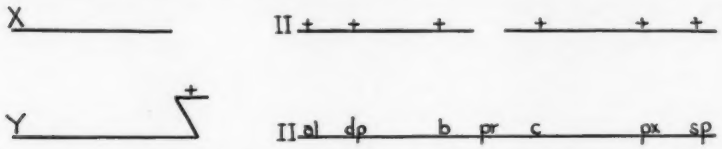
The translocation, with which this paper is concerned, was produced by irradiation with X-rays. Wild-type Oregon males were rayed for 60 minutes with a current of 5 m.a. and a potential of 50 k.v. at a distance of 16 centimeters from the target (for details see papers by DOBZHANSKY) and mated to $\frac{+\ +\ C_y\ +\ p_r\ +\ +\ +}{a_l\ d_p\ +\ b\ p_r\ c\ p_x\ s_p}\ \frac{e_y}{e_y}$ females. (C_y-Curly, p_r-purple, a_l-aristaless, d_p-dumpy, b-black, c-curved, p_x-plexus, s_p-speck are all second chromosome factors, and e_p-eyeless is a gene located in the small fourth chromosome. The "$C_y p_r$" chromosome carries inversions that suppress nearly all crossing over.) Among the F_1 offspring, $\frac{+\ +\ +\ +\ +\ +\ +}{a_l\ d_p\ b\ p_r\ c\ p_x\ s_p}\ \frac{+}{e_y}$ males were selected and individually crossed to $\frac{+\ +\ C_y\ +\ p_r\ +\ +\ +}{a_l\ d_p\ +\ b\ p_r\ c\ p_x\ s_p}\ \frac{e_y}{e_y}$ females. One of the F_2 cultures gave the following progeny:

	Females	*Males*
Wild type	0	10
e_y	0	8
$a_l - d_p - b - p_r - c - p_x - s_p$	8	0
$a_l - d_p - b - p_r - c - p_x - s_p\ e_y$	4	0
C_y	0	15
$C_y - p_r$	16	0
$C_y\ e_y$	0	21
$C_y - p_r\ e_y$	11	0
$a_l - d_p - b - c - p_x - s_p$	0	5

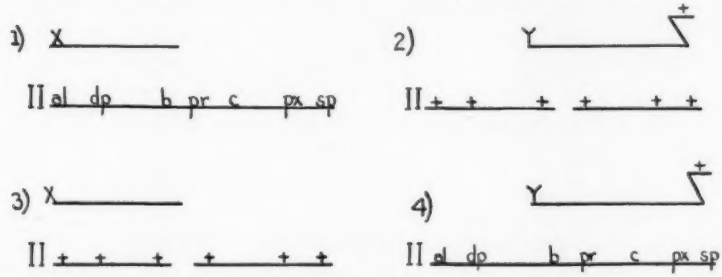
Only 8 of the 16 expected classes appeared, and the data show a coupling or linkage between the irradiated second chromosome, carrying no recessive genes, and the treated Y chromosome. These aberrant results were interpreted as the consequence of a translocation involving the second and Y chromosomes. Further analyses proved the correctness of this interpretation and showed that a small piece from near the middle of the second chromosome, including the locus of p_r, had become fastened to the Y chromosome. The third chromosome was tested and found to be free from the translocation. Some of the wild-type F_2 males were again mated to $\frac{+\ +\ C_y\ +\ p_r\ +\ +\ +}{a_l\ d_p\ +\ b\ p_r\ c\ p_x\ s_p}$ females and among the ensuing F_3 offspring (disregarding C_y flies) were found:

wild-type males	290
$a_1-d_p-b-c-p_z-s_p$ males	30
$a_1-d_p-b-p_r-c-p_z-s_p$ males	4
$a_1-d_p-b-p_r-c-p_z-s_p$ females	255

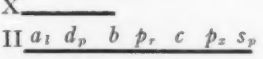
Assuming that the hypothesis of a II-Y translocation is correct, the 30 a_1-d_p-b-c-p_z-s_p and the 4 a_1-d_p-b-p_r-c-p_z-s_p males are unexpected classes whose origin can be explained by the types of gametes formed in the maturations divisions. Let the constitution of the males carrying the translocation be diagrammatically designated as follows:



From the maturation divisions in the male 4 types of gametes are possible (their frequencies being equal only if assortment is random). They are



Since C_v flies are disregarded, the eggs may all be considered as having the same genetic constitution, that is,



Sperm from classes 1 and 2 give rise to regular or expected types of offspring. Sperm from class 4 will produce males, carrying a duplication for a portion of the second chromosome, which show the recessive characters $a_1\ d_p\ b\ c\ p_z\ s_p$ but not p_r since their Y chromosome carries the normal allelomorph or p_r in the attached piece, and 1 normal allelomorph is dominant over 2 recessive genes. The 30 $a_1\ d_p\ b\ c\ p_z\ s_p$ males were of this constitution. A check was afforded by crossing duplication males from similar cultures to

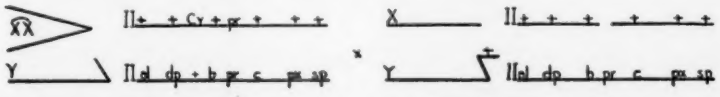
$a_l\ d_p\ b\ p_r\ c\ p_z\ s_p$ females. Only $a_l\ d_p\ b\ p_r\ c\ p_z\ s_p$ daughters and $a_l\ d_p\ b\ c\ p_z\ s_p$ sons should be produced (excepting occasional cases of non-disjunction where sperm carrying both a paternal X and a paternal Y chromosome are formed). Such was found to be the case.

The 4 $a_l\ d_p\ b\ p_r\ c\ p_z\ s_p$ males could result if sperm carrying neither an X or Y fertilized an X bearing egg. Such males would be of XO constitution as they would lack a Y chromosome. They should be sterile, since the presence of a Y in the male Drosophila is necessary for fertility. Although these 4 males were not tested similar males from other cultures were tested for fertility and no offspring were ever obtained.

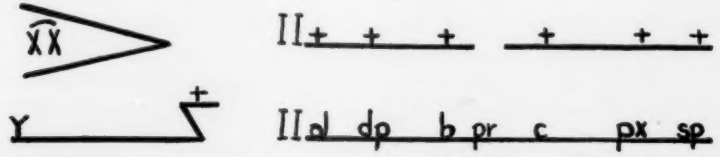
Sperm of class 3 would produce daughters showing only the recessive character purple eye. They would, however, carry a deficient second chromosome and the absence of this class of females among several thousand flies is taken to indicate that the deficiency is lethal although the duplication gives rise to viable and fertile flies of both sexes.

EFFECT OF TRANSLOCATION ON CROSSING OVER IN THE SECOND CHROMOSOME

Since the translocation involved the second and Y chromosomes it was possible to study the effect of the translocation on crossing over in the second chromosome only in attached-X females (females which are XXY in constitution but whose 2 X chromosomes are attached to a single spindle fiber and, therefore, always pass to the same pole). To secure the desired stock the following breeding procedure was followed. Attached-X females carrying the dominant gene C_y and the recessive gene p_r in one of the second chromosomes and the recessive factors $a_l\ d_p\ b\ p_r\ c\ p_z\ s_p$ in the other were mated to wild-type males carrying the translocation. Diagrammatically the procedure was as follows:



(Although the attached-X-'s were homozygous for the factor forked (*f*) only second-chromosome factors will be listed.) The F_1 wild-type daughters were of



constitution and were mated with normal males homozygous for the 7 recessive second-chromosome genes. The data from these crosses are presented in tables 1 and 2 but the summarized crossover values are given below:

Region	*Percents of crossing over in females with translocation*	*Percents of crossing over in control series*	*Difference*
1 a_l–d_p	1.9	10.0	− 8.1
2 d_p–b	4.8	29.0	−24.2
3 b–p_r*	0.0	8.9	− 8.9
4 p_r–c	24.6	20.0	+ 4.6
5 c–p_x	24.1	21.1	+ 3.0
6 p_x–s_p	6.7	5.1	+ 1.6

* Crossing over in region 3 calculated from females only.

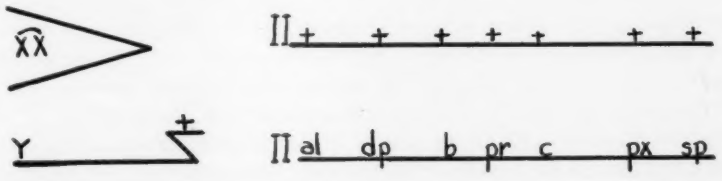
Certain classes of females in table 1 and certain classes of males in table 2 are listed as arising from non-disjunctional gametes. Failure of the attached-X's and the Y (carrying the deleted piece of the second chromosome) to pass to opposite poles in the heterotypic meiotic division in females carrying the translocation would result in (1) eggs with both the attached-X pair and the Y and (2) eggs with neither the attached-X pair or the Y. Fertilization of class 1 eggs by sperm with a normal Y would presumably yield females with 2 Y chromosomes, one with a piece of the second chromosome, and an attached-X pair, although no direct evidence of their constitution exists. Fertilization of class 2 eggs by X-bearing sperm would give XO males, which would be purple eyed, since the normal allelomorph is in that piece of the second chromosome attached to the missing Y. Several of the supposed XO males were tested for fertility and invariably found to be sterile. DOBZHANSKY (1930a and in press) in his analyses of 8 different translocations found similar exceptional males. He had an additional check upon their XO constitution as he crossed translocation females with attached-X chromosomes to normal males whose X chromosomes carried the recessive factor bobbed (*bb*). Since the normal allelomorph of bobbed is located in the Y chromosome, XY males are not-bobbed in appearance while XO males are bobbed as well as sterile. In this experiment the sterility of the purple-eyed males was considered adequate evidence as to their XO constitution.

The data show crossing over to be greatly reduced in the left arm of the second chromosome, the greatest proportional reduction being in the b–p_r region, which included the portion involved in the translocation, where no crossing over took place. Regions 1 and 2 also showed a marked reduction in the amount of crossing over. The amount of crossing over in the right

limb of the second chromosome is consistently higher in all 3 regions than the control. This agrees with the results found by DOBZHANSKY (1929, in press and unpublished data), whose data usually show in II-IV, III-IV and II-Y translocations an increase in crossing over in those limbs of the second and third chromosomes which were not concerned in the translocation.

CROSSING OVER IN FEMALES CARRYING THE DUPLICATION

The above data show that in females carrying the translocation there is a great reduction in the amount of crossing over taking place in the left arm of the second chromosome. It is, perhaps, instructive to consider the causal agents of this reduction in crossing over. Among the several possibilities are (1) that an inversion in the linear order of the genes occurred at the time the translocation was produced; (2) that an injury of some kind to the chromosome prevents or inhibits normal pairing and crossing over; (3) a mechanical interference or hindrance to normal synapsis resulting from the formation of rings or chains during the prophases of the maturation divisions; or (4) an effect produced by a combination of factors. Fortunately, it was possible to study the effect of the duplicated portion of the second chromosome on crossing over between 2 normal second chromosomes since females carrying the duplication were both viable and fertile. Some light might, therefore, be gained on the cause of the decrease in crossing over when a translocation is involved. From an appropriate series of crosses, attached-X females were obtained that had the following constitution



They were mated to normal males homozygous for the 7 recessive second-chromosome factors. The data from this type of cross are given in tables 4 and 5. The summarized percentages of recombination are listed below with the percentages found for the same regions in the translocation and control series.

Region	Duplication percentages	Control percentages	Difference	Translocation percentages	Control percentages	Differences
1 a_l-d_p	10.8	10.0	+0.8	1.9	10.0	− 8.1
2 d_p-b	21.8	29.0	−7.2	4.8	29.0	−24.2
3 $b-p_r$	2.0*	8.9	−6.9	0.0*	8.9	− 8.9
4 p_r-c	20.0*	20.0	0.0	24.6	20.0	+ 4.6
5 $c-p_z$	21.7	21.1	+0.6	24.1	21.1	+ 3.0
6 p_z-s_p	6.0	5.1	+0.9	6.7	5.1	+ 1.6

* Calculated from female offspring only.

The unusual feature of the duplication data is that although crossing over in the right limb is almost exactly of the same order as in the control there is a decided and significant decrease in region 3, which is in the left arm, a proportionately smaller decrease in region 2, and an approximately normal amount of crossing over in region 1. It might reasonably be inferred from these data that at least a portion of the decrease in crossing over in females carrying the translocation is caused by the disturbing influence of that portion of the second chromosome attached to the Y, since a plausible explanation of the effect of the duplicated piece on crossing over between 2 normal second chromosomes is that it pairs with them, the presence of the Y mechanically interfering with normal synapsis. It logically follows that the greatest disturbance would be expected in that region of the chromosome including the duplicated piece with a progressive decrease in effect proceeding away from the duplicated portion. Actually this was realized, since crossing over was normal in region 1, but was greatly decreased in region 3 with an intermediate value in region 2. That the duplicated piece had little or no effect on crossing over in the right arm of the second chromosome is shown by the very close agreement in values with the control.

It would seem that if the reduction in crossing over is caused by the pairing of the duplicated piece with the normal second chromosomes that the distribution of the Y with respect to the attached-X pair might be such that the frequency of non-disjunction (where both the attached-X pair and the Y go to the same pole in the reduction division) would be increased. However, the low frequency of non-disjunctional gametes from females with the duplication indicates that the attached-X pair and the Y nearly always pass to opposite poles. It is apparent, therefore, that the distribution of the Y and the sex chromosomes proceeds in an approximately normal manner even though the reduction in crossing over in the left arm of the second chromosome may be caused by the pairing of the duplicated piece with the second chromosomes. The calculated percentage of non-disjunction is not greater than 3 percent in duplication females. It is impossible to calculate the true amount of non-disjunction since some un-

tested flies might be due either to certain crossovers or to non-disjunction. But the inclusion of all doubtful cases does not give a percentage of non-disjunction greater than 3.

GENETICAL DETERMINATION OF SIZE OF DUPLICATED PIECE

Since duplication flies, that is, flies carrying the piece of the second on the Y in addition to 2 normal seconds, were viable it was possible to determine which genes were included in the duplicated piece by the following scheme. For example, to ascertain whether the locus of rolled wing (r_l) was included, wild-type males carrying the translocation but with the recessive genes black (b) and rolled (r_l) in their normal second chromosomes were mated with $b\ r_l$ females. The regular offspring would consist of $b\ r_l$ daughters and wild-type sons. Duplication sons would be either b, if r_l is included in the extra piece, or $b\ r_l$, if r_l is not included. A recessive factor such as black (b), known to be not included in the duplicated piece was used in testing all loci, except for hooked (h_k), to facilitate the detection of duplication males. In the case of hooked the absence of hooked males was taken to mean that this locus was included in the duplication. The following genes, located near the p_r locus, were tested and are listed below with their current map positions, as determined by BRIDGES:

Locus	*Map position*	*Determination*
Black (b)	48.5	Not included in duplicated piece
Scraggly (r_{d^2})	51.0	Not included in duplicated piece
Hooked (h_k)	53.9	Included in duplicated piece
Purple (p_r)	54.5	Included in duplicated piece
Light (l_t)	55.0	Included in duplicated piece
Rolled (r_l)	55.1	Not included in duplicated piece
Thick (t_k)	55.3	Not included in duplicated piece
Cinnabar (c_n)	57.5	Not included in duplicated piece

Three genes, hooked, purple and light (an allelomorph of pink-wing) were found to be included in that portion from the middle of the second chromosome which is attached to the Y. Genetically the minimum distance included is 1.1 map units and the maximum distance is 4.1 map units. The position of the spindle-fiber attachment is known to be approximately 0.5 of a map unit to the right of purple. Therefore the rightmost end of the translocated section must lie very close to the insertion point of the spindle fiber. The mutant Bristle, whose locus at 54.7 apparently lies in the translocated section, offered difficulties in testing, on account of its dominance, and positive inclusion has not yet been proven.

Cytological examination of ganglion cells from male larvae carrying the

translocation failed to disclose any discrepancy in length between the two second chromosomes, one of which had a deficiency while the other was normal.

SUMMARY

1. From a male which was treated with X-rays a translocation was secured that involved the second and Y chromosomes.

2. Analyses showed that a small piece had been removed from near the middle of the second chromosome, including the loci of the genes hooked (h_k), purple (p_r), and light (l_t) and had become attached to the Y chromosome. The minimum map distance of the translocated piece is 1.1 units while the maximum length is 4.1 map units.

3. Cytological examination failed to show a discrepancy in length between the two second chromosomes, one of which was normal and the other deficient for the translocated piece.

4. Studies of crossing over in females carrying the translocation showed a great reduction in the left arm of the second chromosome and slightly higher values than the control in the right limb.

5. Studies of crossing over in females carrying the extra piece of the second attached to the Y in addition to two normal seconds (that is, duplication females) showed a significant reduction in the left limb of the second chromosome and approximately normal percentages for the right limb. The greatest reduction in crossing over was in region 3, which includes the piece involved in the translocation, with a proportionately lesser decrease in region 2, while region 1 showed about the same amount of crossing over as the control.

6. It is suggested that the Y carried by the translocated piece may interfere with synapsis of the second chromosomes, and possibly may account for a portion of the reduction in crossing over in translocation females, since in duplication females there is a decreased amount of crossing over in the left limb of the second chromosome.

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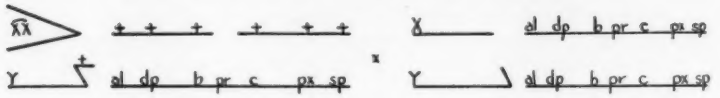
TABLE 1

Translocation data.

Crossing over in attached-X females carrying the translocation.

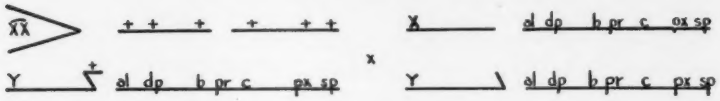


FEMALES ONLY		
$0 = a_l\ d_p\ b\ p_r\ c\ p_x\ s_p$	449	
0 = Wild	8	(non-disjunction)
$1 = d_p\ b\ p_r\ c\ p_x\ s_p$	8	
$2 = b\ p_r\ c\ p_x\ s_p$	28	
$4 = a_l\ d_p\ b\ p_r$	201	
$4 = c\ p_x\ s_p$	2	(non-disjunction)
$5 = a_l\ d_p\ b\ p_r\ c$	206	
$5 = p_x\ s_p$	2	(non-disjunction)
$6 = a_l\ d_p\ b\ p_r\ c\ p_x$	43	
$6 = a_l\ d_p\ b\ c\ p_x$	3	(non-disjunction)
$1-4 = d_p\ b\ p_r$	3	
$1-5 = d_p\ b\ p_r\ c$	4	
$1-6 = d_p\ b\ p_r\ c\ p_x$	2	
$2-4 = b\ p_r$	3	
$2-4 = b$	1	(non-disjunction)
$2-5 = b\ p_r\ c$	8	
$2-6 = b\ p_r\ c\ p_x$	5	
$4-5 = a_l\ d_p\ b\ p_r\ p_x\ s_p$	16	
$4-6 = a_l\ d_p\ b\ p_r\ s_p$	11	
$5-6 = a_l\ d_p\ b\ p_r\ c\ s_p$	2	
$1-4-5 = d_p\ b\ p_r\ p_x\ s_p$	1	
$1-5-6 = d_p\ b\ p_r\ c\ s_p$	1	
$2-5-6 = b\ p_r\ c\ s_p$	2	
$4-5-6 = a_l\ d_p\ b\ p_r\ p_x$	1	
Total	1010	

TABLE 2

Translocation data.

Crossing over in attached-X females carrying the translocation.



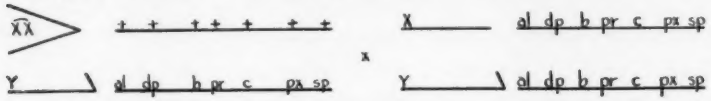
MALES ONLY

0=wild	386	1–5=d_p b c	2
0=a_l d_p b c p_z s_p	289	1–5=a_l p_z s_p	1
0=a_l d_p b p_r c p_z s_p (XO)	4	2–4=b	4
		2–4=a_l d_p c p_z s_p	9
1=a_l	15	2–5=b c	4
1=d_p b c p_z s_p	3	2–5=a_l d_p p_z s_p	6
2=a_l d_p	21	2–6=a_l d_p s_p	2
2=b c p_z s_p	22	2–6= b c p_z	4
4=a_l d_p b	141	4–5=a_l d_p b p_z s_p	9
4=c p_z s_p	179	4–5=c	19
5=a_l d_p b c	143	4–6=c p_z	10
5=p_z s_p	171	4–6=a_l d_p b s_p	7
5=a_l d_p b p_r c (XO)	1	5–6=a_l d_p b c s_p	9
		5–6=px	1
6=a_l d_p b c p_z	30	1–5–6=d_p b c s_p	1
6=s_p	37	2–4–5=b p_z s_p	2
1–2=d_z	1	2–4–5=a_l d_p c	3
1–4=a_l c p_z s_p	5	4–5–6=c s_p	3
1–4=d_p b	1		
		Total	1545

TABLE 3

Control data.

Crossing over in attached-X females carrying 2 normal second chromosomes and a normal Y.



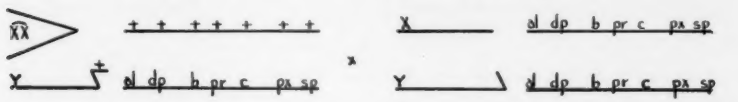
MALES AND FEMALES

0=	436	2–6=$a_l\ d_p\ s_p$	18
0=$a_l\ d_p\ b\ p_r\ c\ p_z\ s_p$	312	2–6=$b\ p_r\ c\ p_z$	17
1=a_l	58	3–4=$a_l\ d_p\ b\ c\ p_z\ s_p$	9
1=$d_p\ b\ p_r\ c\ p_z\ s_p$	63	3–4=p_r	13
2=$a_l\ d_p$	189	3–5=$a_l\ d_p\ b\ p_z\ s_p$	16
2=$b\ p_r\ c\ p_z\ s_p$	193	3–5=$p_r\ c$	21
3=$a_l\ d_p\ b$	52	3–6=$a_l\ d_p\ b\ s_p$	8
3=$p_r\ c\ p_z\ s_p$	55	3–6=$p_r\ c\ p_z$	5
4=$a_l\ d_p\ b\ p_r$	111	4–5=$a_l\ d_p\ b\ p_r\ p_z\ s_p$	17
4=$c\ p_z\ s_p$	119	4–5=c	23
5=$a_l\ d_p\ b\ p_r\ c$	110	4–6=$a_l\ d_p\ b\ p_r\ s_p$	3
5=$p_z\ s_p$	138	4–6=$c\ p_z$	10
6=$a_l\ d_p\ b\ p_r\ c\ p_z$	18	5–6=$a_l\ d_p\ b\ p_r\ c\ s_p$	0
6=s_p	29	5–6=p_z	2
1–2=$a_l\ b\ p_r\ c\ p_z\ s_p$	8	1–2–4=$a_l\ b\ p_r$	1
1–2=d_p	5	1–2–4=$d_p\ c\ p_z\ s_p$	1
1–3=$a_l\ p_r\ c\ p_z\ s_p$	4	1–2–5=$a_l\ b\ p_r\ c$	1
1–3=$d_p\ b$	5	1–2–6=$a_l\ b\ p_r\ c\ p_z$	1
		1–3–4=$a_l\ p_r$	2
1–4=$a_l\ c\ p_z\ s_p$	17	1–3–4=$d_p\ b\ c\ p_z\ s_p$	1
1–4=$d_p\ b\ p_r$	18	1–3–5=$d_p\ b\ p_z\ s_p$	1
		1–3–6=$d_p\ b\ s_p$	1
1–5=$a_l\ p_z\ s_p$	28	1–4–5=$d_p\ b\ p_r\ p_z\ s_p$	1
1–5=$d_p\ b\ p_r\ c$	18	1–4–6=$a_l\ c\ p_z$	2
		1–4–6=$d_p\ b\ p_r\ s_p$	1
1–6=$a_l\ s_p$	3	2–3–4=$b\ c\ p_z\ s_p$	1
		2–3–4=$a_l\ d_p\ p_r$	3
2–3=$a_l\ d_p\ p_r\ c\ p_z\ s_p$	4	2–3–5=$a_l\ d_p\ p_r\ c$	4
2–3=b	9	2–3–6=$a_l\ d_p\ p_r\ c\ p_z$	2
		2–4–5=$a_l\ d_p\ c$	7
2–4=$a_l\ d_p\ c\ p_z\ s_p$	45	2–4–5=$b\ p_r\ p_z\ s_p$	3
2–4=$b\ p_r$	68	2–4–6=$a_l\ d_p\ c\ p_z$	3
		3–4–5=$a_l\ d_p\ b\ c$	1
2–5=$a_l\ d_p\ p_z\ s_p$	61	4–5–6=$c\ s_p$	1
2–5=$b\ p_r\ c$	60	1–3–4–5=$a_l\ p_r\ p_z\ s_p$	1
		1–2–4–5=$d_p\ c$	1
		1–3–4–5=$d_p\ b\ c\ p_z$	1
		Total	2439

TABLE 4

Duplication data.

Crossing over in attached-X females carrying 2 normal second chromosomes and the translocation Y chromosome.



MALES ONLY

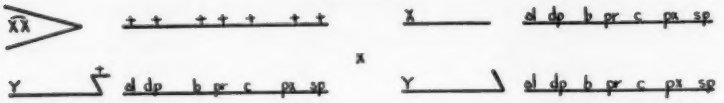
0=wild	259	?2-4=a_1 d_p c p_x s_p	16
0=a_1 d_p b c p_x s_p	120	?2-4=b	17
0=a_1 d_p b p_r c p_x s_p (XO)	1		
		2-5=a_1 d_p p_x s_p	24
		2-5=b c	27
1=a_1	36		
1=d_p b c p_x s_p	7	2-6=a_1 d_p s_p	5
		2-6=b c p_x	8
2=a_1 d_p	52		
2=b c p_x s_p	67	?4-5=a_1 d_p b p_x s_p	7
2=b p_r c p_x s_p (XO)	1	?4-5=c	6
		4-5=a_1 d_p b p_r p_x s_p (XO)	2
3=p_r c p_x s_p (XO)	1	?4-6=a_1 d_p b s_p	2
?4=a_1 d_p b	70	?4-6=c p_x	4
?4=c p_x s_p	55		
4=a_1 d_p b p_r (XO)	1	5-6=a_1 d_p b c s_p	1
		?1-2-4=a_1 b	1
5=a_1 d_p b c	55		
5=p_x s_p	78	?1-4-5=a_1 c	1
6=a_1 d_p b c p_x	10	?1-4-6=a_1 c p_x	2
6=s_p	23		
1-2=a_1 b c p_x s_p	1	?2-4-5=a_1 d_p c	1
1-2=d_p	1	?2-4-6=b p_x s_p	3
?1-4=a_1 c p_x s_p	8	2-5-6=a_1 d_p c p_x	1
?1-4= d_p b	6	?2-4-5-6=b p_x	1
1-5=a_1 p_x s_p	22		
1-5=d_p b c	9		
1-6=a_1 s_p	4	Total	1017
1-6=d_p b c p_x	1		

It is impossible to tell crossovers in region 3 from those in region 4 in the male offspring except in cases of non-disjunction.

TABLE 5

Duplication data.

Crossing over in attached-X females carrying 2 normal second chromosomes and the translocation Y chromosome.



FEMALES ONLY

0=	249	2–6=$a_l\ d_p\ s_p$	6
0=$a_l\ d_p\ b\ p_r\ c\ p_z\ s_p$	116	2–6=$b\ p_r\ c\ p_z$	3
*0=$a_l\ d_p\ b\ c\ p_z\ s_p$	4	†3–4=$a_l\ d_p\ b\ c\ p_z\ s_p$	2
		†3–5=$a_l\ d_p\ b\ p_z\ s_p$	4
1=a_l	46		
1=$d_p\ b\ p_r\ c\ p_z\ s_p$	25	†3–6=$a_l\ d_p\ b\ s_p$	1
2=$a_l\ d_p$	63	4–5=$a_l\ d_p\ b\ p_r\ p_z\ s_p$	6
2=$b\ p_r\ c\ p_z\ s_p$	68	4–5=c	9
		*4–5=$a_l\ d_p\ b\ p_z\ s_p$	1
†3=$a_l\ d_p\ b$	6		
3=$p_r\ c\ p_z\ s_p$	2	4–6=$a_l\ d_p\ b\ p_r\ s_p$	3
		4–6=$c\ p_z$	3
4=$a_l\ d_p\ b\ p_r$	56		
4=$c\ p_z\ s_p$	65	5–6=$a_l\ d_p\ b\ p_r\ c\ s_p$	2
5=$a_l\ d_p\ b\ p_r\ c$	49	1–2–4=$a_l\ b\ p_r$	1
5=$p_z\ s_p$	78		
		1–2–5=$a_l\ b\ p_r\ c$	1
6=$a_l\ d_p\ b\ p_r\ c\ p_z$	6	1–2–5=$d_p\ p_z\ s_p$	2
6=s_p	20		
		1–3–4=$a_l\ p_r$	1
1–2=$a_l\ b\ p_r\ c\ p_z\ s_p$	0	†1–3–4=$d_p\ b\ c\ p_z\ s_p$	1
1–2=d_p	1		
		1–4–5=$d_p\ b\ p_r\ p_z\ s_p$	1
1–4=$a_l\ c\ p_z\ s_p$	8	1–4–6=$d_p\ b\ p_r\ s_p$	1
1–4=$d_p\ b\ p_r$	8		
		1–5–6=$d_p\ b\ p_r\ c\ s_p$	1
1–5=$a_l\ p_z\ s_p$	5		
1–5=$d_p\ b\ p_r\ c$	8	2–4–5=$a_l\ d_p\ c$	3
1–6=$a_l\ s_p$	4	2–4–6=$b\ p.\ s_p$	1
1–6=$d_p\ b\ p_r\ c\ p_z$	5	2–4–6=$a_l\ d_p\ c\ p_z$	1
†2–3=b	1	†2–3–6=$b\ s_p$	1
2–4=$a_l\ d_p\ c\ p_z\ s_p$	8	1–2–4–5=$a_l\ b\ p_r\ p_z\ s_p$	1
2–4=$b\ p_r$	22	†2–3–4–5=$a_l\ d_p\ p_r\ p_z\ s_p$	1
2–5=$a_l\ d_p\ p_z\ s_p$	13	Total	1014
2–5=$b\ p_r\ c$	21		

* non-disjunction.

† not tested for non-disjunction.

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